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GROWTH AND SURVIVAL OF BACTERIA IN PEAT

I. POWDERED PEAT AND RELATED PRODUCTS[1]

BY A. G. LOCHHEAD[2] AND R. H. THEXTON[3]

Abstract

Comparative tests of various powdered materials showed that well humified peat was superior to other preparations in maintaining the test bacteria in viable condition. Under the test conditions, sterilization of peat improved it as a medium for the maintenance of high numbers of viable cells. Neutralization was important, and more satisfactory following, than preceding, sterilization. Potassium carbonate was more suitable than sodium carbonate as neutralizing agent for promoting longevity of bacteria. Though peat allowed to dry immediately after inoculation did not permit the same high increase in bacterial numbers observed in peat kept at suitable moisture content, it was able to maintain large numbers of organisms in viable condition. Peat allowed to dry following the initial rise continued to support an increased load of living cells. After the initial increase at growth temperatures, peat stored at 4° C. maintained numbers of bacteria better than at the higher temperature. Though immediate storage at 4° C. resulted in a pronounced decline in numbers, subsequent removal to a temperature suitable for growth, even though delayed for as long as 12 wk., was followed by a rapid increase of bacteria to very high numbers.

Introduction

The question of employing suitable carriers for bacteria or other microorganisms is often an important matter in industrial and agricultural microbiology. Though in the majority of industrial fermentation processes an inoculum for larger scale cultivation can be suitably prepared in liquid media prior to use, in other cases it is of advantage or even imperative to have the organism maintained in such condition that it may be used after a period of storage to provide an effective inoculum that can be used without further manipulation. This is of importance, for example, in such diverse applications as seed inoculation with various species of *Rhizobium*, the seeding of cheese curd with *Penicillium roqueforti*, or the application to soil of bacteria pathogenic for larvae of destructive insects, as illustrated by the use of *Bacillus popilliae* for the control of the Japanese beetle (2).

From the standpoints of convenience in handling and effective dispersal of the inoculum throughout the material to be treated, a dry carrier has many advantages over a liquid inoculum. Liquid cultures, moreover, have notor-

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iously poor longevity, the viability and effectiveness of the organism being adversely affected by accumulated metabolic products. They are, therefore, not well adapted as carriers requiring storage. The preparation of cultures in lyophile form, though excellent for the purpose of preservation, is less suited to use in larger scale inoculation procedures than cultures maintained in a carrier adapted to more effective dispersion for immediate use.

Various materials have been used as dry carriers for bacteria. Considerable success has been attained in the use of dried skim milk powder as carrier for lactic and propionic acid bacteria for use in dairy manufacturing processes, following the work of Rogers (6). Talc and calcium carbonate have been used by Dutky (1, 2) as carriers for spores of *B. popilliae* in testing the value of this organism in controlling soil-inhabiting insect larvae. In recent years considerable interest has been shown in developing legume seed inoculants consisting of different species and strains of *Rhizobium* carried on sand, soil, charcoal, or humus-rich material such as peat, with the carrier prepared in varying degrees of fineness and with varying moisture content. Though not all have proved efficient, certain inoculants, particularly those of the humus type, have supplanted to a large extent the older, but effective, agar cultures, in view of the greater ease of application.

In studying the longevity of non-spore-forming bacteria in air-dry soil, Giltner and Langworthy (3) concluded that survival is due, not only to retention in soil of moisture in hygroscopic form, but also to the presence, in the richer soils, of soluble organic constituents that have a protective influence on bacteria subjected to desiccation. From such considerations even more favourable conditions for survival might be expected to obtain in peat and related substances rich in organic matter in various stages of decomposition. Moreover, the high adsorptive capacity of such materials suggests a further value for use as carriers through their capacity to immobilize, at least partially, by-products of bacteria metabolism. An additional advantage lies in their relatively light weight, making them, in finely powdered form, adapted to more thorough mixing or covering of the material to be inoculated.

The present report includes comparative tests on the suitability of different grades of peat and lignite as carriers for bacteria, with special attention to the former. Supplies were furnished through courtesy of the Division of Fuels, Department of Mines and Resources. The peat was obtained from subsurface layers of the Alfred, Ont., deposit and represented humified sphagnum that was air-dried and ground for use. It was dark brown in colour. The lignites, darker in colour than the peat, were obtained from Northern Ontario deposits.

Experimental

To compare the suitability of various carriers, preliminary tests were made on the following materials:

Charcoal—fine powder (No. 24)

Earthy lignite—fine powder ('54% minus 200 mesh')

Woody lignite—fine powder ('57% minus 200 mesh')
Peat—fine powder ('48% minus 200 mesh')
Peat—medium fine ('minus.1/32 in. mesh')
Peat—medium coarse ('minus 1/16 in. mesh')
Peat—coarse ('on 1/16 in. mesh')

The moisture content, pH value, and bacteria counts of the air-dry materials as received are shown in Table I. The finer powders could take up 30% or more of moisture and still remain in 'dry' condition. The object of the

TABLE I

MOISTURE, pH, AND BACTERIA COUNTS OF AIR-DRY CARRIERS

	Moisture, %	pH	Bacteria count per gm.	
			Nutrient agar, 28° C., 3 days	Soil extract agar 28° C., 10 days
Charcoal	4.0	8.5	175,000	350,000
Earthy lignite	9.6	6.4	27,000	157,000
Woody lignite	9.6	5.0	190,000	172,000
Peat—fine	11.1	4.6	55,000	165,000
Peat—medium fine	11.1	4.5	130,000	225,000
Peat—medium coarse	11.0	4.4	48,000	49,000
Peat—coarse	9.7	4.8	32,000	41,000

experiments was to determine the numbers of viable organisms on inoculated carriers after periods of storage, and the tests included studies of the effect of sterilization, neutralization, holding temperature, desiccation, and various addenda to the materials. The high adsorptive capacity of peat and related materials for by-products of microbial growth suggested their value, not only as carriers proper (i.e. by mixing with high numbers of organisms, which it is desired to maintain), but as a medium for development of the inoculated bacteria, thereby simplifying the manner of preparation.

As test organisms, non-sporing bacteria, not indigenous to soil or peat, and therefore presumably not specially favoured over autochthonous soil types, were selected. *Salmonella typhimurium* and *Escherichia coli* were used, these organisms having the advantage of being adapted to ready quantitative estimation by plating methods on selective media. It was also felt that results from tests with these species would serve as a starting point for studies on a wider variety of organisms that might be found suited to use in the control of fungal or insect pests.

For analysis 10-gm. samples of material (usually in duplicate) were weighed out aseptically and transferred to a 100 ml. Erlenmeyer flask containing 90 ml. of sterile tap water and fitted with a rubber stopper. The flask was then shaken for five minutes and further serial dilutions were prepared as required and shaken one minute. Appropriate dilutions were transferred to Petri

plates (in triplicate) and plates poured with violet red bile agar (Difco). For *E. coli*, plates were counted after incubation for 24 hr. at 37° C., and in the case of *S. typhimurium*, after 48 hr. This medium was found very suitable for both organisms, giving characteristic dark colonies. At frequent intervals colonies were picked from the plates and compared with the original culture to assure identity.

Comparison of Various Carriers

In the preliminary test 10 carriers were compared—charcoal, earthy lignite (neutralized), and four grades of peat, each of which was tested without being neutralized and after neutralization with sodium carbonate. The materials were first sterilized by autoclaving at 15 lb. pressure for four hours. Neutralization was done by addition of sufficient sodium carbonate to adjust the pH to 6.8 to 7.0. In the case of fine peat with pH of 4.5, 15 ml. of 20% sodium carbonate solution per 100 gm. of peat was usually satisfactory. (Later tests indicated that potassium carbonate was superior to sodium carbonate.) Extra water required to bring the moisture content to 30% was added, allowance being made for the moisture added in the inoculum. For this and most tests a 48 hr. culture of the organism in nutrient broth was added, usually 15 to 20 ml. per 100 gm. of dry powder, and well mixed with the carrier, which was then ready for storage.

Comparative tests of carriers adjusted to 30% moisture, inoculated with *E. coli*, packed in screw-capped containers and stored at room temperature, are summarized in Table II. The results show the superiority of the neutralized peat over the other carriers, and particularly the effect of neutralization on the suitability of peat itself (Fig. 1). Charcoal and lignite, though permitting increases in bacteria up to two weeks, were less satisfactory than the neutralized peats, the numbers failing to reach such high levels and showing an earlier decline.

TABLE II

COMPARISON OF VARIOUS CARRIERS FOR BACTERIA

(INOCULATED WITH *Escherichia coli*, ROOM TEMPERATURE STORAGE, MOISTURE 30%, BACTERIA COUNTS IN MILLIONS PER GRAM)

Length of storage	Charcoal	Earthy lignite	Fine peat		Medium fine peat		Medium coarse peat		Coarse peat	
			Not neutr.	Neutr.	Not neutr.	Neutr.	Not neutr.	Neutr.	Not neutr.	Neutr.
At start	36	36	36	36	36	36	36	36	36	36
1 day	270	125	8.7	330	14	140	19	320	14	650
2 weeks	500	325	1.2	1160	5.4	1290	16	650	22	360
1 month	320	280	1.2	920	7.9	790	18	230	23	780
2 months	115	117	1.5	570	6.3	690	4	140	13	230
4 months	17	10	0.09	210	1.3	205	10	90	9.8	130
6 months	4	3.4	0.008	3.7	0.05	6.1	0.3	3.1	0.2	3.5
9 months	0.7	0.3	0.0003	0.3	0.01	0.7	0.1	0.03	0.03	0.03

A further comparative test was made with the finely powdered materials used as carriers for *S. typhimurium*. Charcoal, earthy lignite, woody lignite, and peat, all neutralized and with a moisture content adjusted to 25%, with

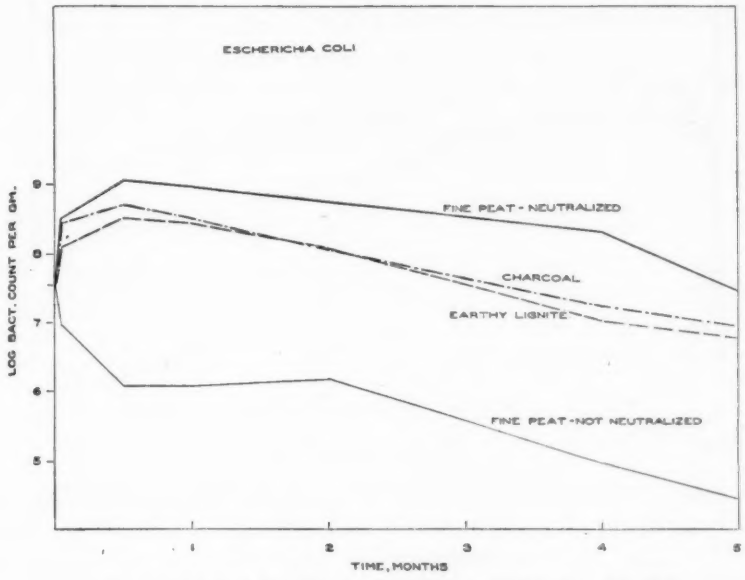

FIG. 1. *Survival of Escherichia coli on various carriers in powder form.*

an additional lot of peat with 30% moisture, were held at room temperature for six months in closed containers. The results (Fig. 2) again showed peat to be superior to the other carriers. With an original count of 26 and 24.5 million cells per gram respectively, the two peat carriers gave maximum numbers of organisms between two weeks' and one month's storage, with viable counts of 36.2 and 21.4 millions per gram after six months' holding. In view of the superiority of peat over the lignites and charcoal, subsequent experiments were confined to peat.

Effect of Moisture Content

Further information on the effect of the moisture content on the development and survival of bacteria added to powdered peat was obtained in a test with *E. coli*. Different portions of the carrier were prepared to contain 20, 25, and 30% moisture respectively and after inoculation were stored at room temperature in closed containers. As indicated in Fig. 3, little difference was noted between lots packed at 25 and 30% moisture (cf. also Fig. 2). At 20% moisture, however, conditions for preliminary increase were less satisfactory and numbers of viable organisms remained at lower levels.

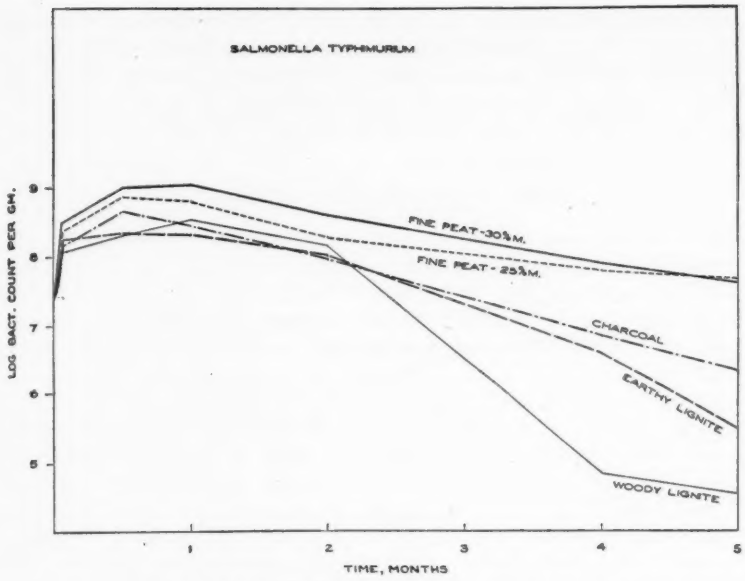

FIG. 2. *Survival of Salmonella typhimurium on various carriers in powder form.*

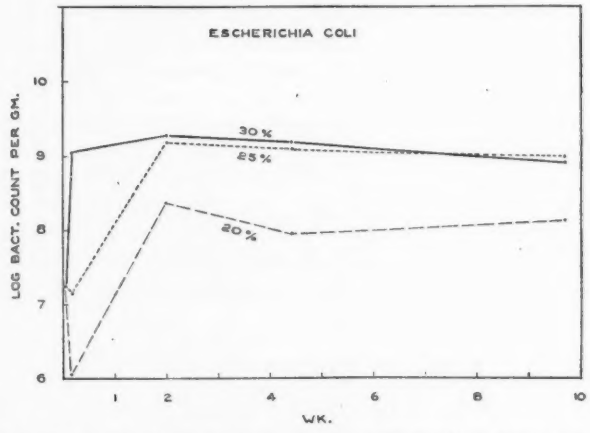

FIG. 3. *Effect of moisture content on numbers of Escherichia coli in powdered peat.*

Effect of Age of Inoculum

To note the effect of the age of the culture used for inoculation, a number of comparative tests were made with fine peat inoculated with 24-hr. and 48-hr. cultures respectively of *S. typhimurium*. Results are illustrated by data

from two tests summarized in Table III. The findings indicate that under the experimental conditions the age of the inoculum was unimportant with the test organism used.

TABLE III

EFFECT OF AGE OF INOCULUM ON SURVIVAL OF *Salmonella typhimurium* IN FINE PEAT

(BACTERIA COUNTS IN MILLIONS PER GRAM)

Storage	Stored at 15°–20° C.		Stored at 26° C.	
	Age of inoculum, hr.			
	24	48	24	48
At start	36	19	17	19
After 1 week	690	600	900	1140
After 2 weeks	610	510	1540	1500
After 3 weeks			1670	1090
After 4 weeks	485	490	1480	1460

Effect of Addition of Milk, Gelatine, or Agar

In the procedure adopted, the only addition of bacterial nutrient material to the carrier was that contained in the inoculating fluid. To test the effect of incorporating other substances, an experiment was made in which skim milk (0.5%), gelatine (0.05%), and agar (0.025%) were added respectively in dilute solutions to the carriers. The results, shown in Fig. 4, indicated that under the conditions of the test the incorporation of these materials was without significant effect.

Effect of Sterilization and Neutralization

Various observations have indicated that humus contains substances that may be injurious or favourable to the growth of higher plants and also of certain micro-organisms (7). Though little of an exact nature is known of such substances, it is believed that they may be liberated, not only as transformation products of the decomposition of plant residues, but also as products synthesized by micro-organisms or formed following their death and eventual decomposition. Lochhead and Chase (5) have shown that soil contains heat-stable growth factors required by certain groups of soil bacteria, some of which growth-promoting substances may be synthesized by other soil bacteria having simpler nutritional requirements. On the other hand soil may contain substances toxic to bacteria, some of which may be destroyed by heat. Among such antibacterial substances are those earlier described as bacterio-toxins (4) and those recognized as related to antibiotics, following the demonstration by Waksman and Woodruff (8) of the occurrence in soil of substances of the actinomycin type. Since growth-promoting and growth-inhibiting substances may both be present, experiments were made to note the effect of sterilization

on the effectiveness of fine peat as a carrier. A summary is presented in Table IV, which includes results from comparative tests with potassium carbonate and sodium carbonate as neutralizing agents.

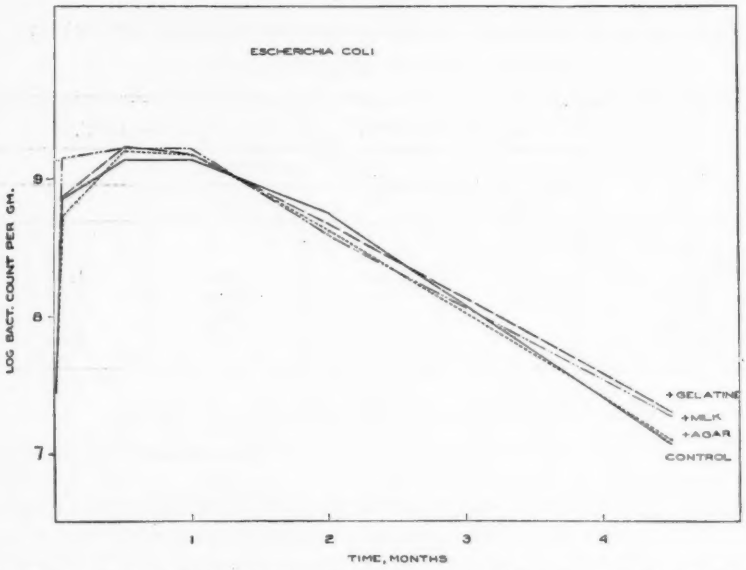

FIG. 4. *Effect of adding gelatine, milk, or agar to powdered peat as carrier for Escherichia coli.*

From these, as well as other tests, the data indicate that sterilization (autoclaving) of the powdered peat used improves it as a medium for supporting growth of certain bacteria introduced into it. To what extent this effect is to be ascribed to a liberation of substances favouring growth, to the removal of toxic substances, or to the destruction of antagonistic micro-organisms is uncertain. Table IV shows also that neutralization should follow, rather than precede, sterilization, and that potassium carbonate is superior to sodium carbonate as neutralizing agent. This was noted also in smaller scale tests in which peat was inoculated with *E. coli*.

Effect of Desiccation

Certain conditions under which bacterial carriers might be used, such as the direct application to soil for pest control, would make them subject to desiccation through exposure to air in thin layers. To test the viability of *S. typhimurium* under such conditions, an experiment was made in which inoculated fine peat, exposed to air in a shallow layer of approximately $\frac{1}{2}$-in. depth, was compared with similar material kept in closed tins containing approximately 400 gm. of peat. A third lot was held for one week in a closed

TABLE IV

EFFECT OF NEUTRALIZATION AND STERILIZATION ON SURVIVAL OF *Salmonella typhimurium* IN FINE PEAT

(BACTERIA COUNTS IN MILLIONS PER GRAM)

Storage period	Temp. of storage	Sterilized, neutralized with K_2CO_3	Sterilized, neutralized with Na_2CO_3	Not sterilized, neutralized with Na_2CO_3	Neutralized with Na_2CO_3 before sterilization
At start	15°–20° C.	35	41	41	41
1 day		80	84	28	49
1 week		180	390	146	178
2 weeks		1060	460	160	27
4 weeks		1130	245	82	10
6 weeks		990	225	70	—
At start	26° C.	19	19	19	
1 day		202	69	71	
1 week		1300	1140	1070	
2 weeks		2290	1500	950	
3 weeks		1570	1090	650	
4 weeks		1840	1460	590	
At start	20° C. ±	33	47		
2 days		1280	700		
1 week		1670	630		
2 weeks		3130	730		
3 weeks		2450	710		
4 weeks		1620	470		
5 weeks		2330	480		
7 weeks		1870	155		
10 weeks		1470	100		

container to allow for a preliminary increase in bacterial numbers before being exposed to air in a shallow layer. Data for bacterial counts and moisture throughout a 10 wk. storage period at room temperature are shown in Fig. 5.

Though immediate exposure to drying prevented an initial rise in numbers comparable to that in the unexposed peat, considerable numbers of viable organisms remained at a comparatively even level. However, when peat was kept for one week after inoculation in the closed containers before being exposed to desiccation, much higher numbers of bacteria were not only reached, but also maintained throughout the 10 wk. period. Though the moisture content decreased rapidly, and became identical with that of the peat exposed immediately after inoculation, no corresponding reduction in counts to similar levels was noted (530 million as compared with 68 million, viable count per gram, after 10 wk.). The results, though indicating that dried powdered peat at low moisture content was less suited to the increase of bacteria, demonstrated a rather remarkable ability of the dried carrier to maintain in viable condition large numbers of bacteria, and to continue to carry an increased load of bacteria in living condition.

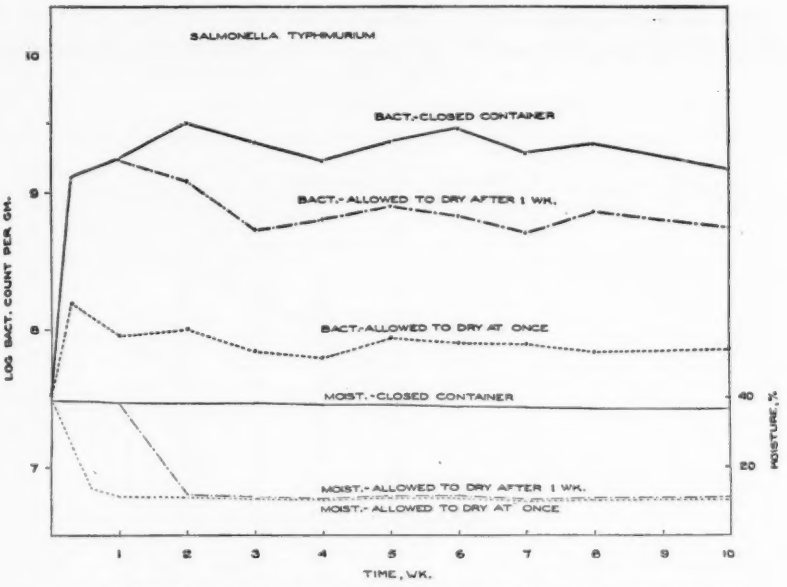

FIG. 5. *Effect of desiccation on survival of Salmonella typhimurium in powdered peat.*

Studies on Storage Temperature

When powdered peat of suitable moisture content is inoculated with *S. typhimurium* or *E. coli* and held at moderate temperatures, there is normally a rapid increase in numbers which reach their peak usually within one or two weeks, after which a gradual decline sets in. A series of tests was undertaken to note the effect of altering storage temperatures so as to provide for higher numbers of bacteria in older material. The tests included comparative studies of:

(a) Continuous storage at room temperature (approx. 20° C.).

(b) Continuous storage at low temperature (4° C.).

(c) Preliminary storage at room temperature for two weeks (to allow for initial increase), followed by storage at 4° C.

(d) Preliminary storage at low temperature (before normal increase has taken place) for various periods, followed by storage at room temperature.

Data for experiments with *S. typhimurium* covering a period of 14 wk. and with *E. coli* during 10 wk. storage are presented graphically in Figs. 6 and 7. It is noted that whereas at room temperature there is a characteristic rapid increase, storage at a temperature of 4° C. shows no such rise, so that numbers of organisms are at all times much lower. It is of interest to observe, however,

that after two weeks at room temperature, removal to storage at the lower temperature results in a slower decline in numbers than continuous holding at room temperature.

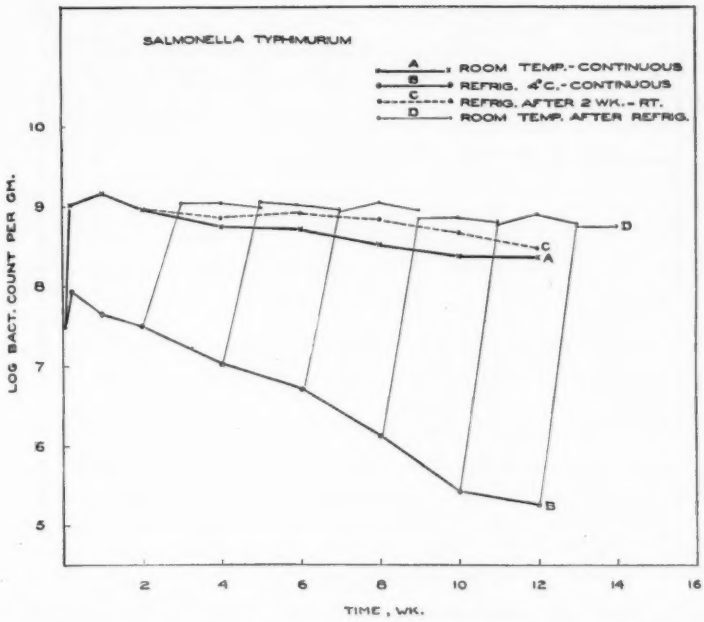

FIG. 6. *Temperature storage conditions in relation to survival of Salmonella typhimurium in powdered peat.*

Results from tests on samples placed at room temperature after varying periods at 4° C. are of special interest in showing that the capacity for rapid increase at suitable growth temperature is well maintained during storage at low temperature for the duration of the experiments. As a result, refrigerated samples brought to room temperature may contain, as in the case of *S. typhimurium*, considerably higher numbers of viable bacteria at any given time than other samples of the same age. This ability to increase rapidly to high numbers (in the region of 10^9 per gram) was shown by samples in which refrigeration had reduced the original count to a comparatively low figure (e.g. 180,000 cells of *S. typhimurium* per gm. after 12 wk., or 185,000 cells of *E. coli* per gm. after eight weeks). This confirms general observations made throughout the studies suggesting that within fairly wide limits the number of organisms in the inoculum is not an important factor in determining the extent to which bacteria will increase. When numbers have fallen below a minimum for good recovery, as indicated by a count of 2000 cells of *E. coli* per gm. after 10 wk. at 4° C., subsequent increase at 20° C. is less

pronounced, though numbers rise to 170 million per gram after one week's transfer to the more favourable temperature (cf. Fig. 7).

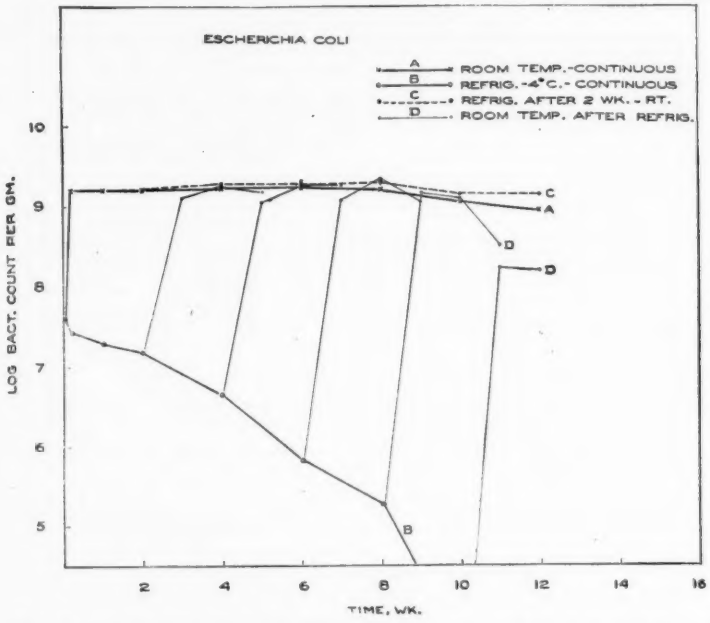

FIG. 7. *Temperature storage conditions in relation to survival of Escherichia coli in powdered peat.*

Growth Promoting Properties of Peat

Since, as has been referred to above, humus-rich material such as the well-decomposed peat employed may contain substances that may exert either favourable or unfavourable effects on the growth of plants or micro-organisms, it is probable that the effectiveness of peat as a medium for promoting growth and longevity of bacteria will vary with the specific organisms under test. In view of the apparent value of the type of powdered peat used in permitting relatively large numbers of the test bacteria to remain viable during storage, some tests were made to note the effect of peat extracts on bacterial development.

For the tests, a synthetic liquid medium was prepared containing mineral salts and sodium citrate as used in Simmons' citrate agar. In this basal medium were incorporated increasing quantities of peat extract, different series of tubes being inoculated uniformly with cultures of various organisms. Two extracts of peat were used, prepared respectively by autoclaving peat with water and by extracting with water without heating. Before use the extracts were brought to neutrality with sodium hydroxide.

TABLE V

EFFECT OF EXTRACTS OF PEAT ON GROWTH OF VARIOUS BACTERIA

Treatment of peat	Test organism	Medium	Time (hr.)	Peat extract per 10 ml. medium				
				0	0.05	0.1	0.5	1.0
100 gm. peat + 300 ml. water autoclav., filt., and neutralized	*S. typhimurium*	Minerals–citrate	24	++	++	+++	++++	
	S. schottmülleri	Minerals–citrate	24	++	+++	+++	++++	
Cold water extract (100 gm. peat + 200 ml. water) neutralized	*S. typhimurium*	Minerals–citrate	24	++	++	++	++	++
	S. schottmülleri	Minerals–citrate	24	+	+	+	++	++
	Aerobacter aerogenes	Minerals–citrate	24	++	++	++	++	++
	Micrococcus sp. (soil)	Minerals–citrate	24	++	+++	+++	++++	++++
	Corynebacterium sp. (soil)	Minerals–citrate	24	++	+++	+++	++++	++++
	Bacterium sp., (Mn-oxidizing-soil)	Minerals–citrate	72	+	+	+++	+++	+++

From Table V it is noted that the addition of peat in the amounts used exerted a generally stimulating effect on the growth of the test organisms, with no depressing action observed. Although the tests were limited in scope they suggest that for some species of bacteria well humified peat contains growth promoting substances. Further investigation is required to elucidate their nature.

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GROWTH AND SURVIVAL OF BACTERIA IN PEAT

II. PEAT PELLETS[1]

By A. G. Lochhead[2] and R. H. Thexton[3]

Abstract

Pellets prepared from spongy, unhumified peat, were found to be well suited to the growth of the test organisms and were superior to pellets made from compressed, well humified peat. Sterilization had the effect of lowering somewhat the absorptive capacity of the spongy pellets and increased the buffering capacity. Neutralization was essential for sterilized pellets in maintaining viable organisms, though it was much less important with unsterilized peat. In contrast to powdered, humified peat, pellets of spongy peat showed to best advantage when allowed to become air-dry immediately following inoculation. In pellets subjected to immediate desiccation, the test organisms attained a much greater development, and were maintained in greater numbers than in peat kept moist or when desiccation was delayed.

Introduction

In a previous report (1) it was shown that powdered peat, prepared from the more compact, well humified layers of a sphagnum bog deposit, was superior to certain other powdered materials as a carrier for maintaining the test bacteria in viable condition for considerable periods of time. In view of the possibility that a material in the form of small pellets might possess certain advantages over fine powder by affording better protection to bacteria exposed to desiccation, additional studies were made with different types of peat in pellet form.

Experimental

Tests were conducted with pellets prepared from two types of peat, as follows:

(*a*) Hard, compressed peat, prepared from well humified material from which the powdered peat previously studied was obtained.

(*b*) Spongy peat from unhumified surface deposits, light brown in colour, slightly compressed but very light and porous.

For the experiments, the hard peat was sawn, and the spongy peat cut, into cubes of approximately $\frac{5}{16}$ to $\frac{3}{8}$ in. (Fig. 1). The cubes of hard peat weighed approximately 0.3 gm. each, and those of spongy peat, 0.05 to 0.06 gm., and gave pH values, when ground up, of 4.7 and 3.8 to 4.3, respectively, the latter being less homogeneous in texture.

Neutralization was done by soaking the pellets in potassium carbonate solution followed by a rinsing in water, after which they were inoculated by immersion for one hour in a 24 hr. culture of the bacteria. They were then

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PLATE I

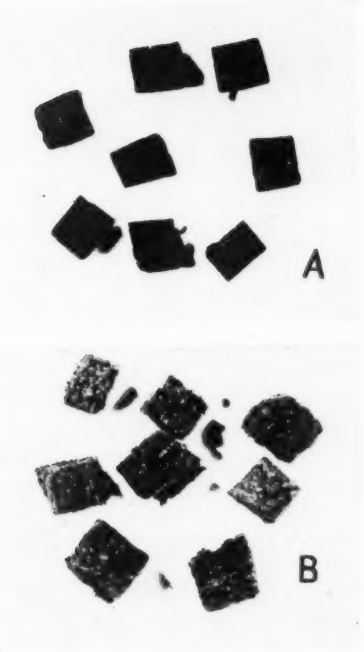

FIG. 1. *Peat pellets: A, hard pellets, prepared from compressed, humified peat; B, soft pellets, from spongy peat.*

allowed to drain and then placed in storage. The test organism was, in most cases, *Salmonella typhimurium*.

For analysis, 5 to 10 pellets were shaken in sterile tap water and appropriate dilutions plated out, in triplicate, with violet red bile agar. This procedure was felt to be more suitable, from the standpoint of practical significance, than grinding the pellet, giving a measure of the bacteria removable by shaking. After 48 hr. incubation at 37° C. plates were counted and numbers of bacteria estimated per pellet or per gram.

Comparison of Hard and Spongy Pellets

Preliminary experiments were made with the two types of peat, the tests being restricted to a study of the effect of immersing the pellets, prior to inoculation, in various solutions to note any influence upon the numbers of viable organisms removable by shaking. Before the treatments the hard pellets were 'neutralized' to pH 6.8 by soaking them in 5% potassium carbonate solution for 40 min., and the spongy pellets immersed in 1% potassium carbonate for 20 min. The pellets were allowed to remain for 16 hr. in the respective test solutions, after which the solutions were drained off and replaced by the inoculum.

The results of several experiments, illustrated by data summarized in Table I, did not provide any significant findings as far as the treatments were concerned. Further tests with such treatment were therefore dispensed with. However, the spongy peat pellets showed to considerable advantage over the hard peat; doubtless this was attributable to the much greater porosity of the former, increased surface, and greater absorptive capacity. In the case of the hard peat, the numbers of viable organisms removable by shaking had

TABLE I

EFFECT OF VARIOUS TREATMENTS OF PEAT PELLETS ON NUMBERS OF BACTERIA REMOVED BY SHAKING

(*S. typhimurium*, STORAGE AT 26° TO 30° C.)

Treatment	Hard pellets. Bacteria removed by shaking, per pellet (millions)				Spongy pellets. Bacteria removed by shaking, per pellet (millions)			
	Days				Days			
	2	7	14	21	2	7	14	21
Glycerine	1040	530	80	13	4560	830	195	84
Dextrin	503	190	55	38	1980	1080	286	435
Glycerine + dextrin	1410	950	183	43	4270	1020	295	49
Dextrin + K_2HPO_4	370	196	12	7	—	—	—	—
Sodium glycerophosphate	—	—	—	—	2180	980	380	273
Gum tragacanth	—	—	—	—	1180	705	250	198
Cysteine + growth factors	337	238	15	5	—	—	—	—
Water	530	145	45	19	870	1100	470	290

declined to figures lower than those considered desirable for a bacterial carrier. Consequently further tests were confined to the spongy peat.

Effect of Sterilization and Neutralization

The exact control of pH in spongy peat presented some difficulty due to the fact that the material was not homogeneous, but still fibrous in nature. It was also noted that sterilization resulted in a somewhat lowered capacity for absorbing liquid. Unsterilized pellets were able to absorb approximately 15 times, and sterilized pellets about 10 times, their weight of water. On drying, moisture was rapidly lost, the pellets becoming air-dry after three or four days and retaining, in this state, about 8% moisture.

Sterilization was found to increase considerably the buffering capacity of the spongy peat. For unsterilized pellets, immersion for 20 min. in 0.5% potassium carbonate solution was found to be sufficient for neutralization, while after sterilization an exposure of 60 min. in 1% solution was required. It was also noted that neutralization of unsterilized peat of this type was not required for the development of *S. typhimurium*, the simple addition of the broth cultures used as inoculum being sufficient to bring the pH to a point

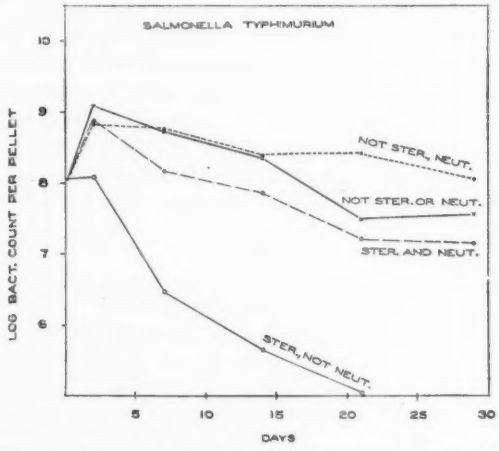

FIG. 2. *Neutralization and sterilization of spongy peat pellets in relation to survival of Salmonella typhimurium.*

suitable for the organisms (pH = 7.0 approximately, after two days). On the other hand, sterilized peat was not similarly affected (pH = 5.4 approximately, after two days), and required neutralization to provide conditions suitable for growth. The relation of sterilization to neutralization in affecting the survival of organisms carried on spongy peat is illustrated in Fig. 2.

Further data on the effect of sterilization and neutralization are shown in Fig. 3. Comparative tests were made with *S. typhimurium* and *Shigella*

alkalescens under conditions that permitted more rapid desiccation of the inoculated material than in the previous experiments. The numbers of viable bacteria are expressed on the 'per gram' basis, permitting a better comparison of the spongy unhumified peat with powdered humified peat as carrier, previously reported (1). Higher numbers of viable organisms (in excess of 10^{10} per gram) were found in the spongy peat than in powdered peat

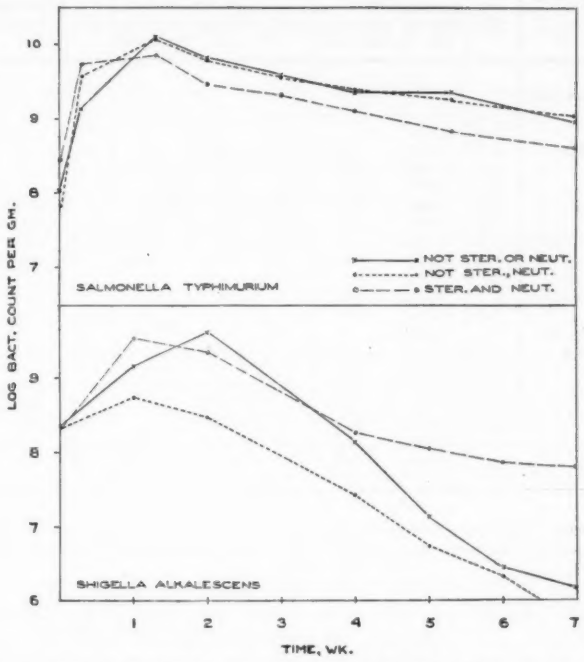

FIG. 3. *Survival of Salmonella typhimurium and Shigella alkalescens on spongy peat pellets.*

within storage periods of three weeks. After storage for seven weeks, the numbers of *S. typhimurium* compared favourably with those found in the powdered material, the unsterilized samples, whether neutralized or not, being as suitable as the sterilized and neutralized peat. *Shigella alkalescens* showed poorer viability than *S. typhimurium*, though in the sterilized and neutralized pellets counts in the neighbourhood of 10^8 per gram were recorded after six to seven weeks.

The importance of sterilization doubtless varies, not only with the organism being carried, but also with the nature of the contaminants in the raw material. Though the use of an unsterilized carrier may be, at first thought, repellent, it should be kept in mind that in larger scale production strict bacteriological sterility is difficult to achieve and maintain. In addition, there is some

TABLE II

EFFECT OF DESICCATION ON NUMBERS OF VIABLE BACTERIA IN PELLETS OF SPONGY PEAT (*S. typhimurium*, STORAGE AT 26° C.)

Treatment and storage	Allowed to dry immediately after inoculation			Closed containers			Closed containers one week, then allowed to dry		
	pH	Average weight, gm.	Bacteria per gram (millions)	pH	Average weight, gm.	Bacteria per gram (millions)	pH	Average weight, gm.	Bacteria per gram (millions)
Not sterilized or neutralized									
Fresh pellets	3.8	0.056	—	—	—	—	—	—	—
After inoculation	5.0	0.747	132	5.0	0.747	132	—	—	—
Stored 2 days	6.5	0.456	2280	6.9	0.713	1420	—	—	—
Stored 1 wk.	7.1	0.057	12,280	7.6	0.719	2380	—	—	—
Stored 2 wk.	6.8	0.056	3800	8.0	0.656	1030	6.9	0.053	1530
Stored 3 wk.	6.4	0.055	2510	7.8	0.638	72	6.4	0.052	680
Stored 4 wk.	6.3	0.057	760	7.8	0.653	0.63	6.5	0.052	114
Stored 5 wk.	6.2	0.057	300	7.6	0.530	0.15	6.1	0.050	8.6
Sterilized and neutralized									
Fresh pellets	3.8	0.056	—	—	—	—	—	—	—
After ster. and neutr.	6.9	0.346	—	—	—	—	—	—	—
After inoculation	6.7	0.522	470	6.7	0.522	470	—	—	—
Stored 2 days	7.4	0.265	2450	7.2	0.533	1760	—	—	—
Stored 1 wk.	7.1	0.051	6690	7.5	0.547	2670	—	—	—
Stored 2 wk.	7.1	0.050	2960	8.2	0.541	1550	7.0	0.050	1420
Stored 3 wk.	6.4	0.051	2670	8.1	0.529	64	6.7	0.049	670
Stored 4 wk.	6.3	0.049	810	7.3	0.564	0.69	6.6	0.045	155
Stored 5 wk.	6.2	0.049	990	7.8	0.511	0.04	6.5	0.047	38

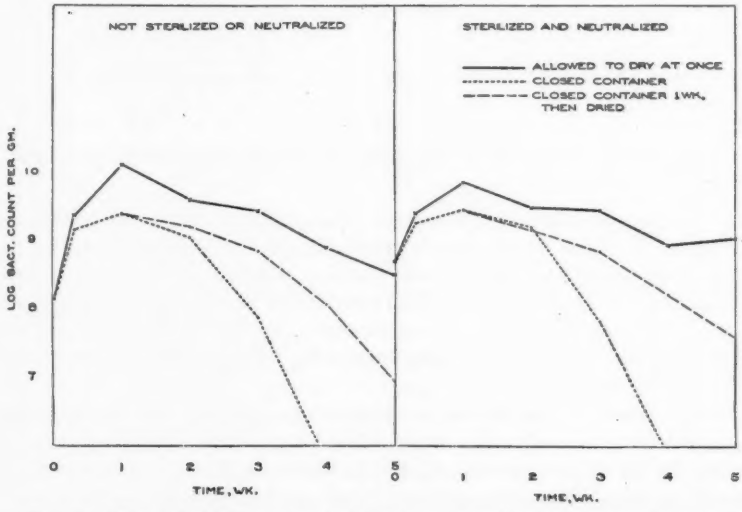

FIG. 4. *Effect of desiccation on survival of Salmonella typhimurium on spongy peat pellets.*

evidence suggesting that 'sterilization' may make the medium more suited to the growth of certain micro-organisms that may be present as heat-resistant types or contaminants. In an experiment in which total counts as well as *S. typhimurium* counts were made with sterilized and unsterilized pellets kept without special protection from contamination following inoculation, the spread was found to become wider with the sterilized pellets.

Certain inoculants for *Rhizobium* of the 'humus' type are not sterile prior to inoculation, but may contain large numbers of actinomycetes and spore-forming bacteria. Since these groups are known to include species that may be highly antagonistic to other bacteria, the presence of such forms may be a factor in determining the value of sterilization in a given case.

Effect of Desiccation

To study more closely the effect of drying on the ability of spongy pellets to carry bacteria, comparative tests were made in which pellets were stored under different conditions:

(i) Allowed to dry immediately after inoculation.

(ii) Kept in closed containers.

(iii) Kept in closed containers for one week, then allowed to dry.

The tests were carried out on two lots (*a*) not sterilized or neutralized, and (*b*) sterilized and neutralized prior to inoculation with *S. typhimurium*. The relevant data, including pH values and average pellet weights, are summarized in Table II while for comparison the viable bacteria counts, in terms of organisms per gram, are presented graphically in Fig. 4.

In both the 'unsterilized' and 'sterilized' series, results were similar in showing the advantage of allowing the spongy peat to dry immediately after inoculation. Not only were higher numbers of organisms per gram obtained (maximum 1.2×10^{10}) but the counts were much better maintained during subsequent holding. Storage for one week in closed containers, followed by drying, was much less satisfactory for promoting longevity of the inoculated bacteria, though much superior to continuous storage in closed containers.

It is of interest to note the pronounced difference in the effect of immediate desiccation on the capacity of spongy peat to carry bacteria, as compared with that of powdered humified peat, as previously reported (1). In the latter case, immediate exposure to drying following inoculation did not show to the same advantage. With the powder it was necessary to prevent drying sufficiently to permit a preliminary increase in bacterial numbers. After this increase numbers were maintained at constantly higher levels than when the carrier was allowed to dry immediately.

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QUALITATIVE STUDIES OF SOIL MICRO-ORGANISMS

VII. THE 'RHIZOSPHERE EFFECT' IN RELATION TO THE AMINO ACID NUTRITION OF BACTERIA[1]

By A. G. Lochhead[2] and R. H. Thexton[3]

Abstract

Comparative studies of the relative incidence of bacteria of different nutritional requirements in soil indicate that one of the most characteristic rhizosphere effects is the preferential stimulation of bacteria requiring amino acids for maximum growth. Organisms for which amino acids are either essential or stimulative were proportionately increased in the rhizosphere. No similar effect was noted with respect to bacteria responding to growth factors.

The findings suggest, by indirect evidence, that the effect is to be ascribed to the excretion of amino acids by the growing plant. However, though this may be the chief factor, the preferential stimulation of the amino acid group of bacteria may be related to associative and antibiotic effects exerted by other bacteria, stimulated in the rhizosphere, observed to have different degrees of compatibility towards those responding respectively to amino acids and growth factors.

Introduction

It is now well established that the growing plant may exert a marked effect, both quantitative and qualitative, on the soil micro-organisms within the zone of influence of the root system (rhizosphere). Soil in the vicinity of the roots supports usually noticeably higher numbers of organisms than soil more distant from the plant, the effect varying with such factors as the kind of crop, the age of the plants, soil treatment, and moisture conditions (1, 3, 5, 11, 12, 14, 15). The rhizosphere of diseased plants may contain greater concentrations of micro-organisms than that of healthy plants (4, 13), while there is evidence that varieties susceptible to certain soil-borne diseases, even though healthy, may show higher numbers of organisms in the rhizosphere than corresponding resistant varieties (15). The 'rhizosphere effect' may be shown, not only in total numbers, but in a preferential stimulation of certain physiological groups of micro-organisms, as judged by quantitative determinations with selective media (2, 3, 5, 7, 8, 11, 12).

Earlier papers in this series (9, 17) have dealt with the influence of plant growth on the characteristics of soil bacteria studied by a quanti-qualitative procedure based on single culture isolations from a non-selective medium, chosen to permit the most nearly representative growth of the indigenous microflora. On the basis of morphology and taxonomic relationship, as well as physiological characters, Lochhead (9) found marked differences in the predominant bacteria in the rhizosphere as compared with soil distant from plant roots, similar trends being noted with all crops studied—clover, oats,

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mangels, corn, tobacco, and flax. In a study of the nutritional requirements of bacteria, West and Lochhead (17) showed that the relative incidence of bacteria for which amino acids and growth factors were essential or stimulative was higher in the rhizosphere of flax and tobacco plants than in corresponding control soils. Using a similar procedure, Katznelson and Richardson (6) found a higher proportion of bacteria stimulated by amino acids in the rhizosphere of tomato plants than in the control soil.

In the nutritional studies referred to, classification of the bacteria was made by observing relative growth in three media, consisting of a basal medium without addenda, and with the addition of amino acids or known growth factors. While these media permit a certain grouping of the organisms, a more complete differentiation, based on nutritional requirements, was provided by the procedure of Lochhead and Chase (10), which involved a determination of growth response in seven cultural media ranging from a simple basal medium to those containing amino acids, growth factors, amino acids with growth factors, or the unidentified substances present in yeast or soil extract or both. This report deals with the rhizosphere effect on the predominant soil bacteria classified on this basis.

Experimental

Bacteria were isolated from control soil and from the rhizosphere of mangels from a four-year rotation system with oats, clover, and timothy. The soil had been maintained at good fertility level by regular application of farmyard manure. For the rhizosphere sample, soil adhering to the roots was used while the control soil was taken midway between rows. Samples were plated on a non-selective soil extract agar without added energy material (3), plates being held at 25° C. for two weeks. Colonies were systematically picked, approximately 180 for each sample, so that all on a plate or sector were taken, and stab inoculation made into soil extract semi-solid (containing 0.02% dipotassium hydrogen phosphate, 0.01% yeast extract, and 0.3% agar).

The nutritional grouping of the isolates was carried out by the procedure previously described (10) involving growth response in all of the following media:

Medium *B* —Basal medium
Medium *A* —Basal medium + amino acids
Medium *G* —Basal medium + growth factors
Medium *AG*—Basal medium + amino acids + growth factors
Medium *Y* —Basal medium + yeast extract
Medium *S* —Basal medium + soil extract
Medium *YS*—Basal medium + yeast extract + soil extract

Nutritional Groups in Rhizosphere and Control Soil

The relative incidence of the various nutritional groups of bacteria in the control and rhizosphere soils, and the estimated number of organisms of the different groups per gram of soil are shown in Table I. The outstanding

TABLE I

NUTRITIONAL GROUPS OF BACTERIA IN RHIZOSPHERE AND CONTROL SOIL

Group	Nutritional requirements for maximum growth	Control soil		Rhizosphere soil		Times increase in rhizosphere
		%	Number per gm.	%	Number per gm.	
	(Plate count)		37,500,000		532,000,000	14.2
I	Grow in basal medium	12.0	4,500,000	22.5	119,700,000	26.6
II	Require one or more amino acids	6.8	2,550,000	25.0	133,000,000	52.2
III	Require growth factors	23.1	8,660,000	15.0	79,800,000	9.2
IV	Require amino acids and growth factors	16.2	6,080,000	15.0	79,800,000	13.1
V	Require yeast extract	16.2	6,080,000	11.7	62,200,000	10.2
VI	Require soil extract	6.8	2,550,000	5.8	30,800,000	12.1
VII	Require yeast extract and soil extract	11.1	4,160,000	2.5	13,300,000	3.2

feature is the increased percentage, in the rhizosphere, of organisms requiring amino acids for maximum growth (Group II), as compared with the control soil. This preferential stimulation is also reflected in the total numbers of this group in the rhizosphere soil, representing a 52-fold increase as compared with a 14-fold increase in total numbers. The table also shows that those groups of bacteria that are dependent upon the more complex nutrients provided by yeast extract and soil extract (Groups V, VI, VII) and not furnished by the amino acids and growth factors, are relatively less abundant in the rhizosphere. This is specially true of those organisms that require for maximum growth certain unidentified substances in both yeast and soil extract (Group VII). On the other hand, those forms that are capable of maximum growth in the basal sugar–salts medium (Group I) are relatively more abundant in the rhizosphere.

Amino Acid Requirements

The relation of amino acid nutrition to the growth of bacteria in the rhizosphere and control soils is presented in greater detail in Table II. A comparison of the growth of the bacteria in the seven media indicated above permits, not only a grouping on the basis of maximum growth, as in Table I, but also a determination of the incidence of those organisms for which amino acids act as essential substances or as a stimulant for growth. In addition to those from the mangels experiment (1945), Table II gives results from earlier rhizosphere studies,* in so far as the relation to amino acids can be calculated from the data. In the earlier work all seven comparative media now employed were not used, but consisted in the 1941 series of Media *B*, *A*, and *G*, and in the 1939 tests of *B*, *A*, and *AG;* thus the same differentiation now possible could not be made.

* *Carried out with the co-operation of P. M. West (1939), and J. J. R. Campbell (1941).*

TABLE II

AMINO ACIDS IN NUTRITION OF BACTERIA IN RHIZOSPHERE AND CONTROL SOIL

—	Mangels (1945)		Flax (1941)		Flax (1939)		Tobacco (1939)	
	%		%		%		%	
	Control	Rhizo.	Control	Rhizo.	Control	Rhizo.	Control	Rhizo.
No growth or submax. growth in basal med.								
Max. growth with amino acids	6.8	25.0	8.4	17.3	4.3	16.5	11.8	13.6
Max. growth, cysteine alone	4.3	13.3	3.1	5.8	—	—	—	—
No growth in basal medium								
Growth (max. or submax.) with amino acids	20.5	30.0	7.3	11.0	14.9	29.1	14.0	24.0
Growth (max. or submax.) with cysteine	13.7	20.9	7.3	10.5	—	—	—	—
Submax. growth in basal medium								
Stimulated by amino acids	10.2	13.3	4.3	14.1	0	14.8	9.6	13.1
Stimulated by cysteine alone	6.0	8.3	4.3	8.8	—	—	—	—
Submax. growth in Medium *G*								
Stimulated by amino acids	17.9	20.8	—	—	—	—	—	—

From Table II it is seen that in all cases there is a preferential stimulation in the rhizosphere of those bacteria for which amino acids provide maximum growth, are essential for growth, or stimulate growth. This is believed to be one of the most characteristic rhizosphere effects as far as the nutritional characteristics of soil bacteria are concerned.

Growth Factor Requirements

Analogous data with respect to the growth factor nutrition of bacteria in rhizosphere and control soils are shown in Table III with the inclusion of

TABLE III

GROWTH FACTORS IN NUTRITION OF BACTERIA IN RHIZOSPHERE AND CONTROL SOIL

—	Mangels (1945)		Flax (1941)		Flax (1939)		Tobacco (1939)	
	%		%		%		%	
	Control	Rhizo.	Control	Rhizo.	Control	Rhizo.	Control	Rhizo.
No growth or submax. growth in Med. *B* or *A*								
Max. growth with growth factors	23.1	15.0	23.2	17.8	8.6	12.4	19.3	19.3
Max. growth, growth fact. and amino acids	16.2	15.0	—	—				
No growth in Med. *B* or *A*								
Growth (max. or submax.) with gr. factors	15.4	19.2	13.7	7.9	14.9	11.0	17.2	15.6
Growth (max. or submax.) with gr. factors and amino acids	7.7	5.0	—	—				
Submax. growth in Medium *B*								
Stimulated by growth factors	9.4	3.3	7.3	8.4	—	—	—	—
Submax. growth in Medium *A*								
Stimulated by growth factors	20.5	10.0	—	—	4.3	16.7	20.4	26.1

findings from earlier studies as far as the data permit calculation. In contrast to the results for amino acids, no significant 'rhizosphere effect' is noted with respect to the relative incidence of bacteria responding to the growth factors used.

Associative and Antagonistic Effects

Though the equilibrium between various groups of organisms existing in a soil at a given time will depend in large measure upon the availability of nutrients required for the growth of these organisms, associative and antagonistic effects are factors that might well play a role in establishing the microbial balance under a given set of conditions.

As noted in Table I, bacteria of Group I, capable of maximum growth in the basal medium, were relatively more numerous in the rhizosphere than in the control soil, though stimulated less than organisms of Group II. An attempt was made to study the action of Group I organisms on those of Groups II and III, respectively, by noting the effect of the culture filtrates of the first group on the growth of the other groups: (*a*) in the basal medium in which they normally do not grow, and (*b*) in Media *A* and *G*, respectively, to observe antibiotic effect in media in which they normally grow well.

Ten organisms of Group I were inoculated respectively into 50 ml. of the basal medium (*B*). After five days' incubation at 25° C. the cultures were filtered (Seitz) and 0.5 ml. of the respective filtrates added aseptically to different series of tubes each containing 5 ml. of Medium *B* or Medium *A*. Tubes containing filtrate from each of the Group I organisms, as well as control tubes without filtrate, were inoculated with one 1-mm. loopful of a suspension of each of 10 different strains of Group II organisms in respective series and incubated at 25° C. for five days. The tubes were then examined to note the effect of the different filtrates on the growth of each of the test organisms of Group II. Analogous series were prepared with Medium *B* and Medium *G* to note the effect of similar filtrates on Group III organisms.

The results, shown in Table IV, indicate that as far as the organisms tested are concerned, bacteria of Group I not only provide much greater stimulation to those of Group II than Group III, but are much more antagonistic towards Group III. This greater compatibility between Groups I and II than between Groups I and III is shown by a higher percentage of cases in which the filtrates permit Group II bacteria to grow in the basal medium (46.7% as compared with 18.2%), and by a much lower percentage of cases in which antibiotic effects were noted (4.4% as compared with 38.7%).

Discussion

The increased incidence in the rhizosphere of bacteria capable of maximum development in an amino acid medium or stimulated by amino acids raises an interesting speculation as to the relation of this to plant excretions, the exact nature of which is little understood. Though amino acids have been detected in soil and are regarded chiefly as products of the decomposition of organic

nitrogenous residues, the excretion of amino acids by plants was established by Virtanen and Laine (16) who identified aspartic acid and β-alanine in the nitrogenous excretions of leguminous plants. The environmental factors determining such excretion have been thoroughly investigated by Wilson and associates (18). That amino acids may be excreted by non-leguminous plants in amounts sufficient to modify the balance of nutritional groups of bacteria may be postulated.

The formation of amino acids as decomposition products of sloughed-off portions of roots also suggests itself as a possible factor in the rhizosphere effect on the amino acid group of bacteria. Excretion by the root appears more likely, however, inasmuch as the effect may be exerted by the plant in the seedling stage, as was the case in the 1939 and 1941 experiments with flax (Table II).

TABLE IV

ASSOCIATIVE AND ANTAGONISTIC ACTION OF GROUP I ORGANISMS ON BACTERIA REQUIRING AMINO ACIDS AND GROWTH FACTORS (GROUPS II AND III)

Filtrates of Group I bacteria* (Cult. No.)	Group II bacteria**				Group III bacteria***			
	No. of strains tested	No. able to grow in basal med. + filtrate	No. suppressed by filtrate in Med. *A*		No. of strains tested	No. able to grow in basal med. + filtrate	No. suppressed by filtrate in Med. *G*	
			Partially	Completely			Partially	Completely
*C*77	10	3	2	0	11	0	4	0
*R*10	—	—	—	—	11	5	3	1
*R*14	10	4	1	0	11	1	3	4
*R*34	10	2	1	0	—	—	—	—
*R*36	10	3	0	0	11	1	3	1
*R*52	10	8	0	0	11	0	4	2
*R*85	10	5	0	0	11	3	4	0
*R*86	10	6	0	0	11	1	3	2
*R*87	10	6	0	0	—	—	—	—
*R*115	10	5	0	0	11	5	0	0
		% 46.7	% 4.4	% 0		% 18.2	% 27.3	% 11.4
			4.4				38.7	

* *Maximum growth in basal medium.*
** *No growth in basal medium; require amino acids.*
*** *No growth in basal medium; require growth factors.*

The data in Table IV suggest that the preferential stimulation of the amino acid group may be concerned, at least partially, with the phenomenon of associative action between different groups of organisms. This influence, however, may be related indirectly to a shifting of the bacterial equilibrium due to plant excretion, for instance, through a stimulation of Group I bacteria, which might in turn stimulate Group II.

The data here presented furnish indirect rather than direct evidence regarding the mechanism by which the development of special groups of soil bacteria

in the rhizosphere is induced. However, the study of nutritive differences in micro-organisms in the rhizosphere is offered as one useful means of approach in obtaining a better knowledge of the physiological activity of the root system of plants, and of factors concerned with such practical problems as crop rotation and the control of soil-borne diseases of crops.

Acknowledgment

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FACTORS INFLUENCING GROWTH AND SUMMER DORMANCY IN *TARAXACUM KOK-SAGHYZ*[1]

By G. W. Scarth,[2] H. B. Gooding,[3] and M. Shaw[3]

Abstract

A setback in rosette diameter and leaf number was observed in both first and second year *Taraxacum kok-saghyz* plants at a definite stage in the plant ontogeny whatever the time of planting. This condition was evidently not entirely synonymous with the 'summer dormancy' of Russian workers as it did not involve complete loss of leaves, nor endure so long. The length of the 'dormant' period, which occurred at the time of reproduction and could be delayed, although not prevented by removal of flower buds, varied from about two to eight weeks. Various external modifications of environment had no appreciable effect on its onset. Watering and disbudding each increased the total fresh weight yield. Watering affected rosette diameter more markedly than it did root weight; disbudding affected both to approximately the same extent. Shading was not beneficial to growth.

The phenomenon of 'summer dormancy' in *Taraxacum kok-saghyz* is reported in Russian literature to be of the greatest importance in reducing the potential yield of the crop, but the writers do not agree as to the extent to which its incidence can be avoided, especially in the second and subsequent years of growth. Some, e.g., Neiman and Sosnovets (4) believe that the dormancy is entirely dependent on climate; others, such as Lebedeva (2), that it is an unavoidable phase of development. Tikhovskaia (5), from a survey of the literature, concludes that *T. kok-saghyz* and also *T. tau-saghyz* have an inherent tendency to enter a rest period at a definite stage of their ontogeny, but that the time of onset and duration of the rest period are modified by external conditions.

Experience in cultivation of *kok-saghyz* in North America indicates that, despite the high quality of *kok-saghyz* rubber, the crop must give greatly increased yields if it is to be of any economic importance. Accordingly, the present experiment was designed to repeat a study of the factors that are said to reduce dormancy, estimating their effect, not only from ultimate yield, but also from the progress of growth during the critical summer period.

Plan and Method

The experiment was laid down in the Macdonald College Department of Horticulture on a plot provided with a sprinkler system for watering. The soil is a sandy loam.

In order to distinguish internal rhythm from the effects of seasonal change, weather conditions, and other environmental differences, rows were planted

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at different times. Several rows were carried over from the previous year, and various sowings were made from late March to late June of the current year (1944).

Experimental modifications of environment consisted of (1) watering, (2) shading by muslin screens, and (3) removal of flower buds as formed. The watering was applied to half of the plot, that is, to half of all plantings, the other treatments to portions of rows including second year plants and two early sowings of first year plants. Watering was done for an hour each day, except in very dull weather, from the end of May until the beginning of September, the object being to reduce sun-heating of the soil as well as to supply additional water to the plants. The other treatments were applied to both unwatered and watered sections. Shading of second year plants was begun on June 28. Removal of flower buds (hereafter termed disbudding) was started at the beginning of flowering at the end of May and continued until the end of the reproductive period. In addition to notes on development, growth was estimated quantitatively by counting the number of leaves in several rosettes of certain plantings and measuring the diameters of the same and other plants at intervals throughout most of the growing season.

In order to determine the effects on yield, samples representing the various plantings and treatments were harvested on Sept. 21 and 22 and again on Oct. 21. The plants were uprooted carefully with a long spade to avoid severe damage to the root systems. The tops were severed from their roots by a transverse cut just below the crown, each was washed free of soil, air-dried for 5 to 10 min. and then weighed. The roots were dried in a soil sterilizer at 55° to 60° C. until quite brittle and weighed again.

Cultural procedure common to the whole plot included fall ploughing and manuring, watering as required until seedlings appeared or until transplants were established, application of liquid fertilizer on May 31, except to the later sowings, and on June 19 to all the rows, and cultivation and hand picking to keep down weeds. The rows were 18 in. apart, transplants were set 10 in. apart in the rows, and field sowings were thinned with the hoe to small groups of plants at about 6 in. intervals. A variation, which was not part of the original design, was introduced. The earlier field sowings, made at a depth of 1 to 1.5 cm., gave slow and irregular germination, probably due in large measure to the formation of soil crust. Consequently, in later sowings, changes were made to accelerate germination.

Table I summarizes the different plantings and their establishment and survival. Three different seed samples *A*, *B*, and *C* were used, all provided by the Division of Botany, Central Experimental Farm, Ottawa. The range of forms among the plants grown from the different samples did not differ significantly. About 10% were weed species, and these were mostly eliminated in transplanting or thinning.

The table shows that transplantation of seedlings started in the greenhouse was very successful, aided, no doubt, by early watering. Field sowing, at least in a soil that tends to become crusted, requires to be shallow, about

TABLE I

TIMES AND MODES OF PLANTING VS ESTABLISHING OF PLANTS

Year	Transplants				Field sowings				
	Seed sample	Sown in greenhouse	Transplanted	Established	Seed sample	Sown	Depth, cm.	Appearance	Stand
1943	One row *A*	April	May	90%	Several rows	May	1–15	Slow	Very sparse
1944	One row *B*	March	May 9	90%	Two rows *B*	May 12	1–15	18 days	Poor and irregular
	One row *B*	April	May 29	90%	Two rows *B*	June 2 (vernalized)	1–15	11 days	Fairly regular
					One row *C*	June 12	0.5	6 days	Excellent
					One row *C*	June 22	0.5	6 days	Excellent

0.5 cm. Deeper sowing was improved somewhat by vernalization of the seed (soaking for three weeks at 1° to 2° C.). No pretreatment could much improve the stand obtained with untreated seed, when sown at a depth of 0.5 cm. or less in well prepared soil and kept moist until the plants appeared.

Plantings of the previous season suffered little frost killing during the winter, but showed great mortality from root rot during their second summer. Injury began to show about May 15; about 30% of one row was dead by the end of June, and 42% by Sept. 25. The rot appeared to be most common where plants were crowded, this fact suggesting a spread of infection from plant to plant.

Results

A. Internal Cycle of the Plant (Fig. 1)

I. General Behaviour

All the plantings showed a definite developmental cycle, which, within the limits of the respective growing periods, was adhered to regardless of the time of planting. Early growth was characterized by a rapid increase in size as measured by rosette diameter and leaf number. Flowering began shortly before the attainment of maximum size and the period between the peak and completion of flowering marked the beginning of a decline in size. This period of setback will be referred to provisionally as the dormant period. Its relation to 'summer dormancy' referred to in the Russian literature will be discussed later. The dormant period, which varied in length from about two to eight weeks was followed by a period of renewed, though perhaps less intense, activity during which leaf number and rosette diameter rose again fairly sharply. As before, this vegetative growth terminated in a reproductive phase, and again a decrease in size set in before the end of flowering.

While the pattern of the cycle is the same for first and second year plants there are, however, certain apparent differences. The second year plants reached their maximum size by mid June, and flowered continuously from

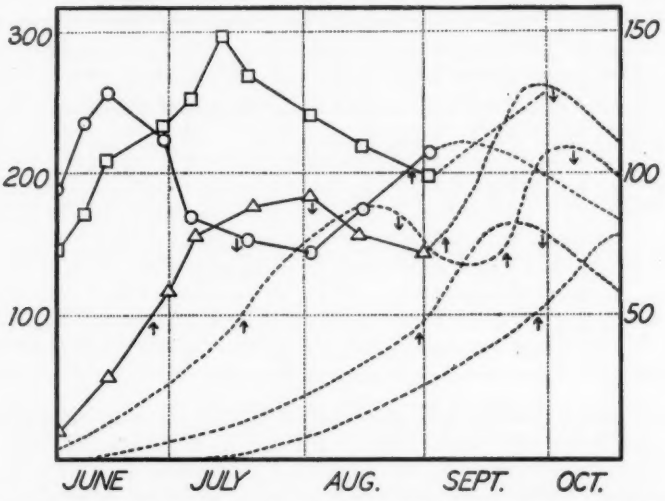

FIG. 1. *Comparison of growth cycles of different plantings. Continuous lines represent actual counts of leaf number plotted from the average value of several plantings: broken lines are hypothetical curves constructed from general observations of May 29 transplants and of the averages of May 12 and June 2 and of June 12 and 22 plantings respectively. The scale for first year is double that for second year plantings.* ○ = *second year.* □ = *second year disbudded.* △ = *first year May 9 transplants.* ↑ = *beginning and* ↓ = *end of flowering.*

May 15 until July 15. Yellowing of the outer leaves was observed as early as May 22 but the decline in leaf number, which lasted for eight weeks and cut the rosettes to one half or less than one half their diameter, began at the height of flowering in mid June. In the first year plants on the other hand, though yellowing of the leaves was noted at the beginning of flowering, the decline in leaf number and rosette diameter did not start until the end of the flowering period, which lasted four to five weeks, e.g., in the earliest planting from the end of June until about Aug. 1. This decline continued only for two or three weeks and did not compare in intensity or in duration with the setback shown earlier in the season by the second year plants. In both first and second year plants the second period of vegetative growth and flowering was less profuse than the first.

As regards root growth the harvesting yields show a decrease in the fresh weight of roots as well as tops between Sept. 21 and Oct. 21 in those plantings that were completing a period of flowering at that time. The decrease of the roots is proportionately much smaller than that of the tops. Thus during dormancy the top/root ratio declines and a low ratio may be taken as a sign of dormancy. Table II shows differences of behaviour as regards growth

TABLE II

FRESH WEIGHT IN GRAMS PER PLANT AND TOP/ROOT RATIO (T/R) AT HARVESTING

Date of harvesting		Sown: Mar.	Apr.	May 12	June 2	June 16	June 22
Sept. 21	Av. wt.	74	52.5	41	33	26.5	16
	T/R	1.1	1.35	2.4	1.85	1.8	2.15
Oct. 21	Av. wt.	69	50.5	39	33	28.5	17.3
	T/R	1.0	1.13	1.45	1.55	1.6	1.25
Wt. change, % Sept. 21 to Oct. 21		−7	−4	−5	0	+8	+9

between Sept. 21 and Oct. 21 depending on the age of the plants, as discussed in the next paragraph.

II. Relation to Time of Planting

The earliest sown first year plants, which were transplanted to the field on May 9, passed through their first dormant period during the first two or three weeks of August, and began their second decline early in October. Root growth ceased after Sept. 21.

The second set of transplants, set out about three weeks later, first showed dormancy during the last week of August and the first part of September. Their root yields on Sept. 21, as compared with those of the first transplants, as well as their higher top/root ratios suggest that they had not completed their second grand period by that time, although by Oct. 21 a decline in top weight seemed to have begun.

The field sowings of vernalized seed made on May 12 and June 2 were noted as beginning to flower about Aug. 30, the average time after germination being about the same as with those started earlier in the greenhouse. Between Sept. 21 and Oct. 21 these sowings showed little change in total weight and a marked decline in top/root ratio, which, in conjunction with their date of flowering, indicates that, flowering having been completed, their rosettes were passing through their first decline in October.

The latest sowings (June 12 and 22) required all that was left of the growing season to pass through the first grand period of their growth. The average diameter of the rosettes of the June 22 sowing increased from 5 in. on Aug. 15 to 7 in. on Sept. 9, the increase continuing through September. At the end of September the plants were just beginning to flower and the weight of the plants increased considerably between Sept. 21 and Oct. 21, as the harvesting results indicate.

Fig. 1 summarizes the growth cycles of the different plantings in graphical form. Only the continuous lines represent actual counts of leaf number. The remainder indicate probable trends as inferred from notes on development,

from diameter measurements of the two latest sowings, and from the fresh weights of the tops at the different harvestings.

In a growing period of seven months the second year plants completed, and first year plants almost completed, two growth cycles. With later sowings and shorter growing periods the development was terminated at progressively earlier stages, but as far as it went showed no essential difference. Since in the different plantings decline occurred in June, July, August, September, or October depending on the age of the plants this is not simply a summer phenomenon. Dormancy tends to occur at a definite stage of ontogeny, but is far more severe in second than in first year plants.

III. Individual Cycles (Fig. 2, Tables III and IV)

In Fig. 1, in which the curves are plotted from the average values for several plants, the dormant period appears to be extended over a longer period of time and to be accompanied by less of a decrease in growth and size than was actually the case. This is due to slight differences between the individual plants.

TABLE III

Leaf number and rosette diameter (in inches) of first year sample plants

Treatment	Rosette measurements	June				July		August			September
		2	9	16	30	7	21	1	15	29	18
Watered	Leaf No.	15	21	38	90	147	190	198	135	105	196
	Diam.	6½	8	11	15	15	14	14	12	7	
	No.	10	15	23	40	53	58	54	41	60	65
	Diam.	4	5	6	8	9	8	6	7	8	
	No.	16	20	26	86	83	90	91	89	130	214
	Diam.	3	3	4½	7	8	9	8	7	8	
	No.	16	24	33	55	74	66	74	51	55	60
	Diam.	6	7	10	12	13	13	12	5	4	
	No.	15	21	33	62	87	110	103	86	118	150
	Diam.	5	6	9	11	12	11	9	10	10	
Unwatered	No.	19	24	30	55	84	102	118	125	121	163
	Diam.	6	9	10	13	14	13	13	12	11	
	No.	18	29	44	88	141	155	120	160	90	100
	Diam.	5	6	6	8	9	9	10	4	3	
	No.	12	17	24	28	48	58	71	67	75	68
	Diam.	4	5	6	9	9	9	9	7	8	
	No.	17	17	36	55	74	67	83	50	36	93
	Diam.	5	6	7	9	10	8	8	2½	3	
	No.	10	13	23	33	44	42	41	33	35	51
	Diam.	4	5	6½	7	9	9	6	5	4	
Unwatered, shaded	No.						77	69	69	69	55
	Diam.						10	12	10	7	
	No.						99	110	165	191	290
	Diam.						12	11	11	10	
	No.						84	98	107	169	205
	Diam.						12	12	11	11	
	No.						68	67	66	71	70
	Diam.						11	11	9	7	
	No.						78	68	75	66	64
	Diam.						7	10	7	6	

TABLE IV

LEAF NUMBER AND ROSETTE DIAMETER (IN INCHES) OF SECOND YEAR SAMPLE PLANTS

Treatment	Rosette measurements	May	June			July			August		
		31	7	14	28	5	12	19	2	16	31
Unwatered	Leaf No.	75	116	136	173	182	181	205	163	110	95
	Diam.	9	12	12	14	12	13	12	10	8½	9
	No.	240	275	330	246	186	85	54	50	95	120
	Diam.	16	16	16	16	12	12	9	3	2	3
	No.	170	220	210	115	80	27	19	23	30	40
	Diam.	15	15	16	15	12	10	9	1	3	4
Watered	No.	179	231	263	208	157	194	208	216	323	374
	Diam.	13	14	13	14	14	12	9	11	10	12
	No.	254	311	304	273	231	268	244	238	265	345
	Diam.	12	13	13	13	13	12	12	9	8	8
Disbudded, unwatered	No.	205	230	220	195	235	260	260	305	290	200
	Diam.	11	11	12	10	10	10	12	12	10	10
Disbudded, watered	No.	118	166	215	324	375	425	391	354	328	340
	Diam.	12	13	14	14	14	14	13	13	10	9½
	No.	219	289	329	375	400	495	520	341	332	316
	Diam.	13	14	15	15	16	16	16	15	12	11

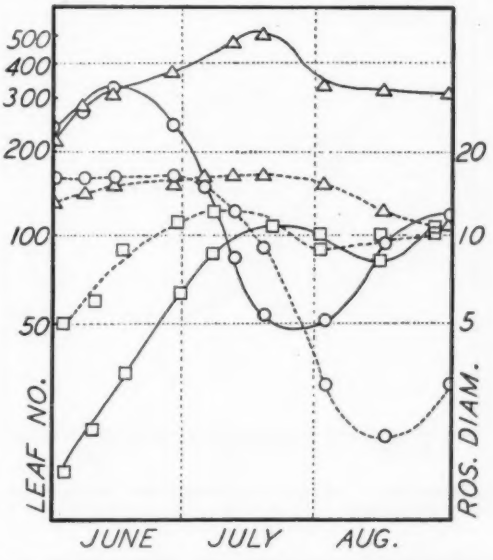

FIG. 2. *Comparison of leaf number and rosette diameter of individual plants. Continuous line = leaf number. Broken line = rosette diameter.* □ *= first year.* ○ *= second year.* △ *= second year disbudded.*

Fig. 2, in which the curves are for typical individual first and second year plants, probably presents a truer picture of the duration and degree of dormancy. From Fig. 2 it is seen that in first year plants reduction in rosette diameter precedes reduction in leaf number by approximately two weeks, whereas in second year plants the reverse is true, and reduction in leaf number occurs approximately two weeks before reduction in rosette diameter. Disbudded second year plants are intermediate in their behaviour; reduction in leaf number is coincidental with reduction in rosette diameter. The relationship between these three types of cycles and its bearing on the nature of dormancy is discussed further below.

B. Effect of External Factors on the Growth Cycle

I. Weather Conditions

The weather from May until September 1944 was characterized by a high proportion of bright hot days and by a low over-all rainfall. Particularly

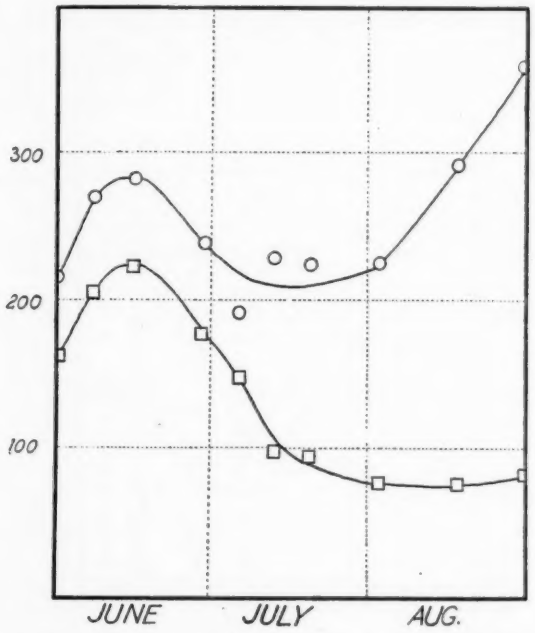

Fig. 3. *Average variation in leaf number of watered and unwatered second year plants.* □ = *unwatered.* ○ = *watered.*

unfavourable conditions prevailed from June 26 until Aug. 17, during which period the temperature and light intensity maintained very high values. If dormancy were induced by unfavourable environmental conditions regardless

of the developmental stage of the plant, all the plants in the experimental plot should have been dormant during this 10 wk. period, as were the earliest plantings; such, however, was not the case. Second year plants entered on a new period of vegetative growth in the middle of the hot spell and those plants grown from seed sown on June 2, 12, and 22 increased steadily in rosette diameter and leaf number from the time of planting until mid August, September, and October respectively. It is evident that while unfavourable conditions of environment may slow the rate of growth, they cannot bring about the changes characteristic of dormancy.

II. Watering

Fig. 3 shows the average variations in leaf number of two watered and three unwatered second year plants during the period June to August. Fig. 4 shows the variation in rosette diameters of first and second year plants during the same period. Tables III and IV present data for leaf number and rosette diameter of first and second year plants.

Watering in no way affected the essential pattern of the developmental cycle and did not prevent dormancy, though the decrease in size may be some-

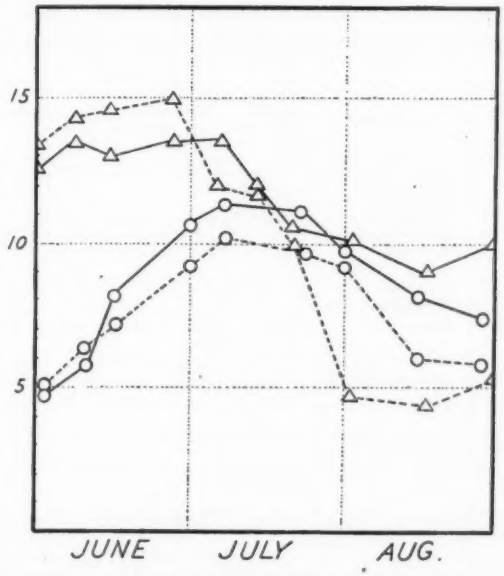

FIG. 4. *Average variation in rosette diameter of first and second year plants.* ○ = *first year.* △ = *second year. Continuous line = watered. Broken line = unwatered.*

what reduced. The main effect was to increase the size of the plants. This is further discussed under yields. Watering may delay the onset of dormancy; the results are not, however, significant.

III. Shading (Table III)

Shading with strips of light factory cotton about 40 ft. long and 3 ft. wide was started in the watered and unwatered second year plots on June 9 and in the first year plots on June 28. The majority of the second year plants under observation were killed by a root rot of an unascertained nature. Observation, however, indicated that while shading may to some extent prevent or lessen the midday wilting, which is particularly noticeable in those plants that have just completed flowering, it does not in any way prevent dormancy in either first or second year plants.

IV. Disbudding (Table IV)

The flower buds were removed from a number of second year plants as soon as they formed. Several of the plants were killed by root rot, but the results show definitely that, though dormancy was not prevented, the onset of the dormant period was delayed by about three weeks. Fig. 1 shows the average variation in leaf number of four disbudded plants. These results confirm those of Lebedeva (2). Disbudding is discussed in relation to yield below.

TABLE V

Yields of first year *Taraxacum kok-saghyz* samples harvested on Sept. 21 and Oct. 21

Sample	Date of field planting	Pretreatment	Field treatment	September				October			
				Fresh weight in gm. per plant			Top: root ratio	Fresh weight in gm. per plant			Top: root ratio
				Total	Top	Root		Total	Top	Root	
40 *B* plants	May 9	Transplants sown in greenhouse end of March	Unwatered	62.0	28.6	33.4	0.85	54.1	22.1	30.0	0.69
			Watered	86.2	50.9	35.3	1.40	85.7	48.0	37.7	1.3
40 *B* plants	May 29	Transplants sown in greenhouse mid April	Unwatered	57.6	32.5	25.1	1.3	59.2	30.5	28.7	1.06
			Watered	47.5	28.3	19.2	1.46	41.8	22.8	19.0	1.2
40 *B* plants	May 12	Untreated seed sown at 1 to 1.5 cm.	Unwatered	32.7	21.0	11.7	1.8	36.6	22.0	14.6	1.5
			Watered	51.1	38.5	12.6	3.05	40.7	23.7	17.0	1.4
50 ft. *B* row, various nos. of plants (40 to 65)	June 2	Vernalized, sown at 1 to 1.5 cm.	Unwatered	31.7	20.5	11.2	1.8	27.5	16.9	10.6	1.6
			Watered	34.0	22.5	11.5	1.9	40.2	24.3	15.9	1.5
				In gm. per foot of row				In gm. per foot of row			
50 ft. *C* row	June 14	Untreated, sown at 0.5 cm.	Unwatered	51.3	33.8	17.5	1.93	53.0	30.9	22.1	1.4
			Watered	54.5	34.0	20.5	1.66	62.5	40.0	22.5	1.78
50 ft. *C* row	June 22	Untreated, sown at 0.5 cm.	Unwatered	24.7	15.6	9.1	1.71	26.3	13.1	13.2	0.995
			Watered	40.0	29.0	11.0	2.64	40.5	24.5	16.0	1.53

From the foregoing it may be concluded that the developmental cycle is due to an internal rhythm that is independent of the environment. The latter cannot change the essential pattern but may exert some modifying effects. Though related in time to the period of flowering it is only postponed and modified by continuous removal of flower buds.

C. Yields

Tables V, VI, and VII summarize the yields of sample plants representing the various experiments. Here the yields are expressed in grams per plant on a fresh weight basis, with the exception of the *C* plantings in which, as the plants were thinned in bunches at 6 in. intervals, they are in grams per foot of row.

I. Relation to Treatment

Total Yield

1. *Watering*

As far as total weight was concerned, watering was found to be beneficial in all first year plants with one exception (Table V). In the May 29 transplants both the September and October yields of unwatered samples were

TABLE VI

Yield of first year samples harvested Sept. 21. The weights are average for 40 plants taken from each group

	Field treatment	Fresh weights in gm. per plant			Top: root ratio
		Total	Top	Root	
Sown in greenhouse end of March. Transplanted May 9	Unwatered	62.0	28.6	33.4	0.85
	Unwatered, shaded	55.0	24.6	30.4	0.8
	Watered	86.2	50.9	35.3	1.4
	Watered, shaded	83.2	53.0	30.2	1.8

TABLE VII

Yields of second year samples harvested Sept. 22

Pretreatment	Sample	Field treatment	Fresh weights in gm. per plant			Top: root ratio
			Total	Top	Root	
Transplants sown in greenhouse March 1943	40 ft. row (=47 plants)	Unwatered	68.5	38.0	30.5	1.3
	40 ft. row (=33 plants)	Watered	64.0	43.5	20.5	2.2
	11 ft. row (=12 plants)	Unwatered, disbudded	86.0	50.0	36.0	1.3
	10 ft. row (=13 plants)	Watered, disbudded	90.0	62.0	28.0	2.2

higher than the corresponding watered ones. No explanation is offered for this discrepancy. Little effect, as a result of watering, was observed in the total weight of second year transplants (Table VII).

2. *Shading*

Data for the effects of shading are presented for a single planting in Table VI. Here shading was found to have a slightly deleterious effect on the growth of May 9 transplants. Confirmatory evidence was obtained from general observations of second year transplants.

3. *Disbudding*

A very marked increase in weight was observed as a result of disbudding of second year plants (Table VII). This greater weight of disbudded plants was undoubtedly due to the longer vegetative period already discussed under factors affecting the cycle and to the prevention of flower and fruit development and the consequent diversion of nutrient material to the vegetative organs.

Root Yield

As most of the rubber is stored in the root the effects of treatment on the root yield are more important from the practical standpoint.

1. *Watering*

Tables V, VI, and VII show that almost invariably the top to root ratio was higher in the watered than in the unwatered part of the plot. This indicates that watering has a more marked effect on the development of the shoot than of the root. However, in first year plantings the root weight was, with the exception of the May 29 transplants, higher in the watered than in the unwatered. The difference was never very large but appeared to be greater in the later sowings than in early ones suggesting that unwatered plants in the later sowings had greater difficulty in establishing their root systems than did early plantings, the roots of which had penetrated deeply before the onset of drier conditions in July and August. The possibility of the greater weight of watered roots being due to a greater succulence was eliminated by a comparison of fresh and dry weight. The fresh to dry weight ratio of a number of samples is presented in Table VIII. Slight fluctuations are probably due to incomplete drying.

Watering definitely retarded root growth of second year plants. It appears likely that this was due to poor aeration of the soil in the watered part of the plot for the soil had not been ploughed for two years and its upper layers often became waterlogged when watered.

2. *Shading*

Root development, as was total development, was hindered by shading. The data are summarized in Table VI.

3. *Disbudding*

Removal of flower buds, as might be expected from a previous discussion, favoured root development. Table VII shows that the increase in weight of roots was proportional to that of tops.

TABLE VIII

RATIO OF FRESH WEIGHT TO DRY WEIGHT OF ROOTS OF FIRST YEAR SAMPLES

Date of planting	Field treatment	Fresh weight/dry weight
May 9	Unwatered	4.22
	Watered	4.42
May 29	Unwatered	4.40
	Watered	4.32
May 12	Unwatered	4.0
	Watered	4.12
June 2	Unwatered	4.13
	Watered	4.04

II. RELATION TO TIME OF PLANTING

Some indication of the importance of early planting on yield is presented in Table IX, where the estimates of root yields in kilograms per acre, on a dry weight basis, of samples of several plantings that were harvested on Oct. 21 are presented. Plants from seedlings that had been germinated in the spring

TABLE IX

DRY WEIGHT ROOT YIELDS OF FIRST YEAR PLANTINGS HARVESTED OCT. 21 FROM UNWATERED PARTS OF THE PLOT

Date of field planting	Pretreatment	Dry weight of roots, kgm. per acre	Av.
May 9	Sown in greenhouse end of March	257	243
May 29	Sown in greenhouse mid April	230	
June 14	Untreated, sown at 0.5 cm.	153	123
June 22	Untreated, sown at 0–5 cm.	93	

in the greenhouse and transplanted later when climatic conditions were favourable gave average yields approximately twice as large as the June plantings. The ratio of the respective lengths of the growing seasons was considerably lower, namely, about 1.65 to 1.0, which reflects the relatively favourable conditions for photosynthesis of kok-saghyz during early summer, reflected also in the steepness of the curves of rosette growth during that period.

Discussion

It has been concluded that in kok-saghyz a recession in rosette size occurs at the end of each grand period of growth. Root weight seems to share to some extent in the decline. While this phenomenon normally begins after the height of flowering, it is not eliminated by removal of flower buds and is therefore better described as the maturation than the reproductive phase of development. External conditions influence the degree and duration of the setback but neither induce nor prevent its occurrence. Though the condition has been referred to as dormancy in the foregoing description, the point at which growth actually ceases is not shown by the type of measurements made in these experiments, and moreover the condition is evidently not entirely synonymous with 'summer dormancy' as described in the Russian papers. The latter involves 'loss of leaves', presumably complete, and is said to begin about mid July and to last one and a half to two months. Under our conditions only a small minority of the plants, all of the second year, suffered to this extent.

Before commenting on the causes contributing to the extreme condition known as summer dormancy, let us consider the interaction of internal and external factors at the very beginning of the decline as revealed by analysis of some of our own results. The order of initiation of the decrease in leaf number and rosette diameter respectively seems to throw some light on the relationship. Second year plants passing into dormancy during the relatively cool weather of June show a marked decrease in leaf number about two weeks before rosette diameter begins to be reduced. This would seem to indicate that the setback begins with a reduction in the activity of the growing point. Similarly the later revival in growth shows an increase in leaf number about two weeks before rosette diameter. On the other hand, in those plants in which the setback occurs during the hot dry spell of July and early August the decline in rosette diameter begins relatively earlier. Thus in the second year plants that had their growth cycle prolonged until this period as a result of disbudding, the decline in rosette diameter begins simultaneously with that in leaf number, while in first year plants it precedes the latter considerably. So also, first year plants protected by shading during the hot weather, if they show any decline at all, follow the same sequence as do the second year plants during the cooler weather. In view of the severe wilting of the outer leaves of plants in the maturation phase during the unfavourable weather, it would seem that their earlier senescence and death was largely caused by an upset in water balance although the high temperatures may also have produced some direct injury. While the immediate cause of the increased death rate of the leaves is thus traceable in part to external factors, the sensitivity to these is due to internal changes since plants in the vegetative phase of development continued to increase in rosette diameter as well as leaf number throughout the testing period.

The greater tendency to loss of leaves in second year plants must also be attributed to internal factors, since in our experiments it was displayed

while the weather was still favourable for growth. These plants, which all flowered heavily, showed a greater percentage reduction of rosettes than flowering first year ones. A qualitative developmental difference between first and second year plants is the 'collar' formation or sloughing off of the old phloem in the roots of the latter. It appears unlikely that there is direct causal connection between this phenomenon and recession of the rosettes. The cork layer that separates the old phloem from the new appears at least a week before the peak of leaf number is reached nor does its formation interrupt the growth of new xylem and phloem in the root. The phenomenon is, however, another evidence of deep-seated developmental change during the flowering phase, and its result, the formation of an almost completely new root, suggests that it may be co-ordinated indirectly with the tendency to defoliation, which results in a more or less new crop of leaves.

It is noteworthy that annual plants in their flowering phase (Loehwing (3)) display the same changes that have been noted for kok-saghyz, namely, reduction or cessation of growth in shoot and root, reduced water balance and increased sensitivity, especially of the older leaves, to drought. Other internal changes, as far as they have been determined, are also paralleled in the two cases. As summarized by Krotkov (1) these include hydrolysis of proteins and carbohydrates, increase in osmotic pressure, and translocation toward reproductive organs and roots. Loehwing distinguishes the flowering from the fruiting phase in annuals and reserves the term maturation for the latter, but in kok-saghyz the long overlapping of flower and fruit formation makes it difficult to separate them as two phases, so maturation has here been used to cover both.

Probably many other perennial plants of rosette habit behave in some measure like kok-saghyz. An example, closely allied it is true, is that of an unidentified species of *Taraxacum* grown side by side with the rows of kok-saghyz and measured in the same way. It displayed a similar though less severe set back after flowering.

To sum up, kok-saghyz after the peak of flowering passes into a period, which may be termed the maturation phase, in which growth is greatly reduced, if it does not cease, and sensitivity to unfavourable conditions, at least such as tend to reduce the water balance, is greatly increased. The degree of defoliation and the time that elapses before a new growth cycle begins is greater, due to inherent causes, in the second (and subsequent) years of the plant's life than in the first. It also depends on the severity of the environmental conditions referred to. Hence the more extreme setback termed 'summer dormancy' is commonly confined to plants that have passed their first year, and it requires for its development the coincidence of the maturation phase with a period of high insolation and considerable drought such as occurs only in midsummer. To this extent 'summer dormancy', though not the occurrence of maturation, is determined by environment.

As regards the suitability of the local climate for growth of kok-saghyz, to judge by the 1943 and 1944 seasons it compares favourably with that of the

zone of cultivation of the plant in Russia. Our second year plants developed rather more rapidly, completing their first flowering by the middle as compared with the end of July, and very few of them displayed the degree and duration of dormancy that is described as usual in Russia. The maturation setback, which is important even in first year plants and under our weather conditions, can best be reduced, it would seem, by selection, though cutting off the flower heads is a feasible beneficial treatment.

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THIAMIN IN MANITOBA VEGETABLES[1]

By Florence Connolly,[2] Mary C. Hiltz,[3] and Allen D. Robinson[4]

Abstract

A survey has been made of the thiamin contents of Manitoba grown vegetables. These are of the same magnitude as of those grown elsewhere. The amounts vary with the vegetable and with the variety. There is often a wide range of values within a single variety. The effect of the district where grown is not significant. Cooking causes some destruction of the vitamin, but on an average boiled vegetables retain 63% of their thiamin, 23% is dissolved in the cooking water, and 14% is destroyed. Storage of raw vegetables does not decrease their thiamin contents appreciably. Canning causes losses, these varying with the vegetable and with the time of blanching and processing.

Vegetables—with the exception of dried legumes—are not usually classed among the better sources of thiamin or vitamin B_1. However, since they comprise an appreciable part of the food we consume—more than 28%, according to a recent survey (21)—the amounts they contribute to our intake of the vitamin may well be significant.

Improved methods of assay have made easier the task of finding the amounts in the various foods that make up our diet. As one would expect, the thiamin content of fresh food is not the same as that of the food when it is consumed. This is important in the evaluation of vegetables, only a part of which are consumed when newly harvested and without cooking. Storage conditions, canning, and cooking may be expected to contribute to the destruction of thiamin in them.

A large number of thiamin assays of fresh vegetables are reported in the literature. Some of the most comprehensive reports are included in Table I. Their results have been calculated to micrograms per 100 gm. The data of Booher, Hortzler, and Hewston (2) and of Fixsen and Roscoe (11) are compilations, the other data report the results of original investigations. Assays of fewer vegetables are reported by Conner and Straub (5), Farrell and Fellers (7), Fellers, Esselen, and Fitzgerald (8), Fincke (9), Fincke, Little, Redelings, and Perkins (10), Gleim, Tressler, and Fenton (12), Heller, McCay, and Lyon (16), Meiklejohn (20), Moyer and Tressler (22), and Oser, Melnick, and Oser (24). Their data are of the same order as those of Table I, with two exceptions. Fincke reports values for green peas as high as 740, while Conner and Straub report 147 as the value for squash. All of these data show that one kind of vegetable varies a great deal in thiamin content, and that there is a marked difference between different kinds of vegetables as

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TABLE I

THIAMIN CONTENT OF FRESH VEGETABLES (μGM./100 GM.)

(DATA REPORTED IN THE LITERATURE)

—	Aughey and Daniel (1)	Booher *et al.* (2)	Fixsen and Roscoe (11)	Hamner *et al.* (15)	Lane *et al.* (18)	Nagel and Harris (23)	Pyke (26)
Artichoke	—	225–228	—	—	—	—	171–285
Asparagus	—	177–195	—	—	—	—	—
Bean, green	66	66	—	46–102	44–94	70	51– 75
Beet	—	18	—	—	25	—	—
Broccoli	—	99	—	—	—	—	72–102
Brussel sprout	—	171	—	—	—	—	—
Cabbage	—	69– 84	75–420	—	37	—	30–138
Carrot	57– 66	40– 72	180	38– 70	76	127	48–126
Cauliflower	—	90–171	330	—	—	92	45–105
Celery	—	36– 45	Trace	—	—	—	—
Chive	—	—	—	—	—	—	114
Corn	—	120–198	—	—	—	—	—
Cucumber	—	45– 54	—	—	—	—	—
Garlic	—	—	—	—	—	—	159
Horseradish	—	—	—	—	—	—	69
Kale	—	189	—	—	—	—	45– 81
Leek	—	—	—	—	—	—	96–147
Lettuce	—	69– 87	<48– 90	—	—	—	—
Onion	—	24– 33	120	—	27	—	30– 57
Parsley	—	—	—	—	—	—	171
Parsnip	—	114	—	—	—	—	—
Pea, green	450	399–450	120–300	259–354	—	392	171–327
Pepper	—	60	—	—	—	—	—
Potato	135–150	48–186	<42–180	53– 90	84	188	—
Pumpkin	—	—	30	—	—	—	—
Radish	—	39	18–180	—	—	—	39– 51
Spinach	129	105–129	60–210	67– 86	100	304	81–156
Squash	—	42– 48	30	—	—	69	—
Tomato	—	45–120	120	—	—	—	24– 69
Turnip	—	33– 60	120	—	51	128	30– 45

sources of thiamin. Of the vegetables reported, green peas appear to be the best source. Artichoke, asparagus, and corn are not quite as good. Potatoes and spinach rank below these, but are still better than many other vegetables. None of the vegetables rank as high as some of the better known sources of thiamin like lean pork with 1830 and oatmeal with 810 μgm. per 100 gm. They are more on a par with peanut butter, eggs, chicken, and bananas whose corresponding thiamin contents are 267, 258, 111, and 54. These last six figures represent values reported by Booher *et al.* (2).

There are comparatively little data on the effect of environment on the thiamin content of vegetables. Fincke *et al.* (10) found little variation in the amounts in peas grown in different parts of Oregon during a single season, but did find a significant difference between the amounts in a single variety grown in the same locality in different seasons.

A number of workers have reported losses of thiamin from vegetables due to ordinary cooking procedures. Aughey and Daniel (1) found that as much

as 22% was destroyed and an additional 15% was dissolved in the cooking water. Cooking losses were much the same for boiled pared potatoes, spinach, green peas (cooked with soda), and snap beans. Green peas cooked without soda showed a loss of 20% (11% dissolved in cooking water) while carrots showed no change. Little or no cooking losses for fresh snap beans or for snap beans frozen for 16 months or canned for 16 months were found by Farrell and Fellers (7).

Heller, McCay, and Lyon (16) found losses of from 16 to 64% in large quantity cookery practices. These were lowered when cooking procedures were changed. Nagel and Harris (23) found that as much as 89% of thiamin was lost from food cooked and handled under restaurant conditions. The total cooking and steam table losses averaged 70%. Oser, Melnick, and Oser (24) found that losses varied with the cooking method, being from 6 to 46% in peas. Greater losses were incurred when larger amounts of water were used. They concluded that with improved methods of cooking, losses should be no greater than 10%. Lane, Johnson, and Williams (18) stress the loss of thiamin in the cooking water. Gleim, Tressler, and Fenton (12) found little actual destruction of thiamin, but solution of from 5 to 54% of it. Meiklejohn (20) concluded that steaming of potatoes extracted the greater part of thiamin from them.

There have been some investigations made of the effect of storage on thiamin content of vegetables. Langley, Richardson, and Andes (19) found no decrease in vitamin B_1 potency of raw carrots stored for four months in either cool or warm cellars. Gleim, Tressler, and Fenton (12) report that asparagus and spinach showed slight loss of thiamin when stored at room temperature for 24 hr., but that asparagus showed a loss of 18% and spinach 15% when stored for a week at 0 to 4.4° C.

There is little information available on the effect of commercial canning operations on the retention of thiamin in vegetables. Most studies have been made on home canning operations. Richardson and Mayfield (27) found no loss of the vitamin in carrots canned by the hot pack method in the oven, but found a slight loss when a pressure cooker replaced the oven. Fellers, Esselen, and Fitzgerald (8) found the thiamin retention of four canned vegetables to be: asparagus 72%, peas 60%, lima beans 28%, and spinach 71%. Farrell and Fellers (7) report a loss of 17% of the thiamin in green snap beans canned at the normal pH of 5.3. Fincke (9) observed a general trend toward lower thiamin contents of peas scalded for longer times and at higher temperatures. No significant difference was noted between peas blanched in steam or in hot water at the same temperature for the same length of time.

Surveys conducted by Clifcorn and Heberlein (4) indicate that blanching and processing are critical procedures in canning. Eddy, Kohman, and Carlsson (6) found that the duration and method of blanch are important. There may be further losses of thiamin in the stored canned vegetables. Farrell and Fellers (7) report losses of 45% in green snap beans after 12 months. Guerrant, Vavich, and Dutcher (14) found that high temperatures

of storage caused further destruction of thiamin in canned tomato juice, lima beans, and yellow corn.

A problem of local interest is whether Manitoba grown vegetables contain the same amounts of thiamin as those grown elsewhere, and whether these amounts are affected by the usual cooking procedures. To answer these questions a study has been made of the thiamin contents of Manitoba vegetables and the effects of processing and storage on them. An attempt has been made to study the variation in thiamin content of vegetables grown in seven districts in and close to Winnipeg.

Experimental

Most of the vegetables used were grown in the University garden at Fort Garry. Only these were used in canning, storage, and cooking experiments. Other samples were obtained on the open market and are designated as such. To show the differences due to environment, samples of five vegetables—beans, beets, carrots, peas, and potatoes—were obtained from seven districts—Narol, Middle Church, Springfield, Winnipeg (Fort Rouge), St. James, Fort Garry, and Domain. This represents an area approximately 30 miles from north to south and 15 miles from east to west.

Vegetables from Fort Garry were analysed the same day as picked. Those from other points were collected the day before, wrapped in wax paper, and stored overnight in the refrigerator.

Analyses were made of 23 different fresh vegetables. Several varieties of some of these were tested. The results of these studies are recorded in Table II. The thiamin contents of the selected five fresh vegetables grown in seven different districts are included in Table III. The two values reported for each vegetable in Table III represent assays of duplicate samples taken from one large representative sample. The data for potatoes obtained from Springfield were completely out of line, the difference between the two determinations exceeding all other differences. Since it was not possible to get another sample from this district, owing to the lateness of the season, new values were estimated according to the method of Yates, as described by Goulden (13). These estimated values are included in Table III.

Only one cooking method—boiling—was employed. The amounts of vegetable and water used were such as to provide from two to four average sized servings. The weights of cooked vegetables and cooking water were determined. Each was assayed separately for thiamin. Results are recorded in Table IV.

The effect of storage was investigated with nine vegetables. From 40 to 60 lb. of each of beets, carrots, onions, parsnips, potatoes, and turnips, six heads of cabbage and six fruits each of pumpkin, and squash, were stored under appropriate conditions in the storage houses of the Plant Science Division. Thiamin tests were made just prior to storage. After approximately four months all vegetables appeared to be in excellent condition and

TABLE II

THIAMIN CONTENT OF MANITOBA VEGETABLES

Vegetable	Variety	No. of samples	Thiamin, μgm./100 gm.		Average serving, gm.	Thiamin in average serving, μgm.
			Range	Mean		
Asparagus	Mary Washington	2	63– 64	64	50	32
	Market	2	30– 31	31	50	16
Bean	Round Pod	—	—	—	—	—
	Kidney Wax	4	49– 61	55	100	55
	Market	12	11– 75	62	100	62
Beet	Detroit Dark Red	7	9– 29	14	100	14
	Market	12	6– 31	17	100	17
Brussel sprout	Market	1	—	88	70	62
Cabbage	Danish Ball Head	4	30– 37	33	50	17
Carrot	Red Cored Chantenay	6	13– 26	20	100	20
	Market	9	24– 47	35	100	35
Cauliflower	Market	2	78– 97	88	70	62
Corn	Golden Bantam	2	109–110	110	100	110
Cucumber	Straight 8	2	22– 23	23	50	12
Kale	Market	2	49– 50	50	100	50
Lettuce	Grand Rapids	2	30– 30	30	10	3
	New York No. 12	4	17– 19	18	10	2
Onion	Yellow Globe Danvers	6	9– 29	19	100	19
Parsley	Market	2	57– 61	59	10	6
Parsnip	Hollow Crown	6	57– 83	73	100	73
Pea	Laxton Progress	2	91–102	97	100	97
	Lincoln	2	167–183	175	100	175
	Homesteader	2	183–189	186	100	186
	Market	10	176–280	211	100	211
Pepper, green	King of the North	2	36– 39	38	100	38
Pumpkin	Small Sugar	4	16– 45	31	100	31
Potato	Irish Cobbler	6	81–103	90	150	135
	Warba	6	77– 97	89	150	134
	Market	10	51– 90	68	150	102
Radish	Scarlet Globe	2	6– 7	7	10	1
Squash	Golden Hubbard	4	6– 18	13	100	13
Swiss chard	Lucullus	2	24– 26	25	100	25
Tomato	Market	2	39– 47	43	100	43
Turnip	Purple Top Swede	6	22– 68	45	100	45

Note: Vegetables labelled "Market" are unidentified varieties purchased on the open market.

TABLE III

THIAMIN CONTENTS OF VEGETABLES FROM DIFFERENT DISTRICTS, μGM./100 GM.

Districts	Vegetables									
	Bean		Beet		Carrot		Pea		Potato	
Narol	73	75	31	31	37	39	280	280	67	65
Middle Church	65	56	17	20	47	43	192	196	68	61
Springfield	62	62	23	26	24	25	176	169	60	59
Winnipeg (Fort Rouge)	35	42	7	7	39	37	173	179	79	72
St. James	64	63	6	7	24	24	189	183	83	84
Fort Garry	60	61	9	9	38	37	183	167	72	72
Domain	75	67	14	11	43	35	239	231	51	55

TABLE IV

THIAMIN RETENTION IN COOKING VEGETABLES

Vegetable	Condition	Weight of sample, gm.	Cooking time, min.	Thiamin in sample, μgm.		Thiamin in cooking liquid, μgm.	Distribution of thiamin, %		
				Raw	Cooked		Retained by cooked sample	Dissolved in cooking liquid	Destroyed during cooking
Asparagus	Fresh	110	15	70	38	17	55	24	21
	Fresh	385	15	118	99	10	84	8	8
Beet	Fresh	600	20	148	32	—	22	—	—
Bean	Fresh	400	20	201	111	87	55	43	2
Brussel sprout	Fresh	300	20	263	141	56	54	21	25
Cauliflower	Fresh	300	15	263	94	43	36	16	48
Cabbage	Fresh	400	20	121	45	44	37	36	27
	Stored 4 months	340	25	84	43	37	51	44	5
Corn	Fresh	400	10	438	418	19	95	4	1
Carrot	Fresh	500	15	68	35	30	51	44	5
	Stored 6 months	500	20	125	112	46	90	37	—
	Stored 10 months	300	20	62	41	27	67	44	—
Kale	Fresh	300	30	149	108	38	73	26	1
Onion	Stored 3 months	300	15	85	65	33	77	39	—
	Stored 8 months	300	15	53	31	17	57	32	11
Parsnip	Fresh	300	10	238	149	82	63	34	3
	Stored 5 months	300	15	173	107	47	62	27	11
	Stored 8 months	300	15	245	191	48	78	19	3
Pumpkin	Fresh	500	15	97	83	15	85	15	0
	Stored 5 months	500	20	217	74	38	34	17	49
Pea	Fresh	635	20	613	315	120	51	19	30
Potato, Irish Cobbler	Fresh	300	15	297	235	78	79	26	—
	Stored 4 months	300	17	258	161	38	62	15	23
	Stored 6 months	300	17	258	226	28	88	11	1
Potato, Warba	Fresh	300	15	233	122	32	52	13	35
	Stored 3 months	300	20	273	136	30	50	11	39
	Stored 6 months	300	20	290	243	25	84	8	8
Squash	Fresh	400	10	70	50	13	71	18	11
	Stored 5 months	300	10	24	26	1	105	5	—
Swiss chard	Fresh	400	10	99	70	38	70	38	—
Turnip	Fresh	300	15	202	82	41	40	20	40
	Stored 4 months	300	15	68	61	—	89	—	—
	Stored 6 months	300	20	137	51	32	37	23	40

were analysed again. By the end of March the beets, cabbage, pumpkin, and squash showed signs of spoilage so a second analysis was not made of any of these four. The other stored vegetables were tested again. Storage conditions are detailed in Table V, and the results of the storage tests are reproduced in Table VI.

Canning studies were limited to eight vegetables—asparagus, beans, beets, carrots, corn, kale, green peas, and Swiss chard. Vegetables were canned the day they were picked, according to standard methods of canning for general household usage as outlined by Pfund (25). The jars were stored in the dark at ordinary room temperatures, and analysed from time to time. These data appear in Table VII.

TABLE V

STORAGE OF VEGETABLES

Vegetable	Variety	Amount stored	Date stored, 1944	Storage conditions	Temperature, °F.
Beet	Detroit Dark Red, C.E.F. strain	50 lb.	Oct. 10	Cool and moist (packed in peat)	32–40
Cabbage	Danish Ball Head	6 heads	Oct. 21	Cool and moist	32–40
Carrot	Chantenay	50 lb.	Oct. 12	Cool and moist (packed in peat)	32–40
Onion	Yellow Globe Danvers, Strain No. 11	60 lb.	Sept. 25	Cool and dry	34–45
Parsnip	Hollow Crown	40 lb.	Oct.	Cool and moist	32–40
Potato	Irish Cobbler	50 lb.	Sept. 7	Cool and moist	32–40
	Warba	50 lb.	Aug. 30	Cool and moist	32–40
Pumpkin	Small Sugar	6 fruits	Aug. 28	Warm and dry	58–66
Squash	Green Hubbard	6 fruits	Aug. 28	Warm and dry	58–66
Turnip	Purple Top Swede	50 lb.	Aug. 20	Cool and moist	32–40

TABLE VI

EFFECT OF STORAGE ON THIAMIN CONTENT OF RAW VEGETABLES

Vegetable	Variety	Fresh thiamin content, μgm./100gm.	During storage		After storage	
			Time stored, days	Thiamin content, μgm./100gm.	Time stored, days	Thiamin content, μgm./100gm.
Beet	Detroit Dark Red	12	128	10	—	—
Cabbage	Danish Ball Head	31	121	35	—	—
Carrot	Red Cored Chantenay	14	201	25	292	21
Onion	Yellow Globe Danvers	—	—	28	—	18
Parsnip	Hollow Crown	79	142	58	218	82
Pumpkin	Small Sugar	19	147	44	—	—
Potato	Irish Cobbler	99	121	86	183	86
	Warba	78	107	91	176	97
Squash	Golden Hubbard	18	148	8	—	—
Turnip	Purple Top Swede	67	120	23	182	46

Thiamin was determined by the method of Hennessy and Cerecedo (17). Most duplicate analyses agreed within ± 5%. This is a much smaller difference than that between different lots of the same vegetable or indeed between different vegetables of the same lot. Each value reported is the average of duplicate determinations. Results are reported as micrograms of thiamin per 100 gm. of material.

TABLE VII

RETENTION OF THIAMIN IN VEGETABLES DURING CANNING

Vegetable	Time* of blanch, min.	Time** of processing, min.	Time between canning and assay, days	Retention of thiamin, %
Asparagus	3.5	30	98 321	93 137
Beet	17	30	109 305	19 21
Bean	3	30	111 291	57 47
Carrot	3	30	104 306	109 61
Corn	2	60	125 271	13 13
Kale	3	60	111 250	40 18
Pea	3	45	104 307	29 30
Swiss chard	3	60	98 309	28 24

* *All blanching was done with water at a temperature of 212° F.*

** *Kale and Swiss chard were processed at 250° F. All other vegetables were processed at 240° F.*

Discussion

The results of the analyses of raw vegetables are presented in Table II. The weights for average servings are those of Bowes and Church (3). The amount of thiamin in an average serving is calculated on a raw basis. This is likely more than there would be in an average serving of the cooked or canned vegetable.

The thiamin contents of the vegetables analysed are of the same order as those reported by other investigators. They range from a low of 6 gm. for radish to a high of 280 gm. per 100 gm. for peas. Of those vegetables examined, peas are the best source of thiamin, followed by corn. Potatoes, which are consumed in rather large quantities, are better than most other vegetables studied. Brussels sprouts, cauliflower, and parsnips have somewhat less thiamin than the vegetables mentioned. In some cases—particularly for peas—there is a marked difference between varieties of the same vegetable. There is often a considerable range for a single variety. This is obvious in those cases where a larger number of samples were available for analysis. Other vegetables might be expected to show a like variation had sufficient of them been assayed. In all varieties of beets, onions, and pumpkin studied, the highest value is almost three times that of the low. The amounts

of thiamin in average servings vary as do the amounts of thiamin in the fresh vegetables. The contribution of potatoes is emphasized because of the amounts of them consumed. But certainly those vegetables that are better sources of thiamin are contributing appreciably to our daily intake.

The data of Table III show the thiamin contents of five selected vegetables from seven districts. The actual amounts are similar to those reported in Table II. There is a difference between districts for any one vegetable. However, no one district is uniformly high or low. This point was studied further by making an analysis of variance of these data. The F value for districts was 1.57, which is well below the 5% point of 2.51. This indicates that the thiamin content of the vegetables does not vary significantly over the district studied. There is the possibility that this finding would be different if a larger or more varied district were subjected to a similar survey. Still, our finding is in harmony with that of Fincke *et al.* (10) who worked with a larger area.

The results of the cooking tests appear in Table IV. In each test analyses were made of the raw sample, the cooked sample, and the cooking liquid. The thiamin of the raw sample that is not accounted for in the cooked sample and in the cooking liquid is labelled "destroyed during cooking". Each cooking test is reported separately.

The results show marked differences in the amounts of thiamin destroyed. Several vegetables retain all of their thiamin, either in the vegetable or in the cooking liquid. Some, like cauliflower and turnip, show as much as 40% destruction. Stored pumpkin shows greater destruction than fresh. These increased amounts destroyed do not seem related to cooking times. The results as a whole do not warrant drawing definite conclusions. On the average vegetables retained 63% of their thiamin, and retained as well another 23% in the cooking liquid. The remaining 14% was destroyed. This does emphasize the importance of saving all cooking liquids and using them in such products as gravies, sauces, and soups.

The results of storage tests are recorded in Table VI. This shows thiamin in the fresh vegetables and after varying times of storage. There is no orderly change in the amounts of thiamin. However there does not appear to be any marked decrease in the amounts of the vitamin in the vegetable at the end of six to seven months' storage. Apparent increases are probably due to variability in the vitamin contents of the vegetables themselves.

The data of canning tests are recorded in Table VII. No assays were made on the vegetables immediately after canning. The first ones made on the canned vegetables show in some cases a marked drop in thiamin content. In only two cases do the later retention tests differ appreciably from the first. In each case one of the values is greater than 100%, which must be due to variability in the material itself. It would seem likely that much of the initial drop must have been due to the canning itself.

Beets and corn showed the most severe canning losses. Peas, Swiss chard, and kale exhibited low thiamin retention. Beans showed a retention of 57%, while asparagus and carrots retained most of their original thiamin. Losses were greatest for those vegetables having longer blanching or processing times. On further storage carrots lost some of their thiamin, kale and beets showed a smaller loss, while the other vegetables appeared to retain the balance of the vitamin left after canning.

Acknowledgments

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A SEEDLING BLIGHT AND LEAF SPOT OF FLAX CAUSED BY *CONIOTHYRIUM OLIVACEUM* BON.[1]

By T. C. Vanterpool[2]

Abstract

Coniothyrium olivaceum Bon. was found to be the cause of a seedling blight of flax in 1945 and 1946 in Saskatchewan. Twelve out of 32 seed samples of flax from the 1945 crop selected for their low germination were carrying an average of 8% seed-borne infections. Leaf-spot lesions were found during late August, 1946. The wide distribution of the fungus suggests that it has been present in the province for some time. Hitherto, it has been reported on flax from the Argentine only. A strain of the same organism was present as a trace in one sample of sweet clover seed. Pycnidia develop abundantly in culture. The spores measure 7.5—10 (11.5)μ × 4.2—7.5μ, averaging 9.1 × 6.0μ, which is somewhat larger than the average for the species, but yet within the range limits. The disease is favoured by high moisture and probably by cool temperatures. It is one of the minor diseases on flax.

In June, 1945, *Coniothyrium olivaceum* Bon. was found to be one of the chief fungi associated with a seedling blight of linseed flax growing on summer-fallowed land following crested wheat grass (*Agropyron cristatum* (L.) Gaertn.), at the Dominion Experimental Station, Scott, Sask. *Rhizoctonia Solani* Kühn was present in about equal amounts, and *Fusarium Scirpi* Lamb. and Fautr. var. *acuminatum* (Ell. and Ev.) Wr. less commonly. *C. olivaceum* proved to be moderately pathogenic to flax seedlings in artificial inoculations in the laboratory and in the greenhouse. During the following winter it was isolated from 12 samples of seed from the 1945 flax crop from widely scattered districts of the province. In the spring of 1946, it was isolated from blighted seedlings from three separate localities, and during the latter part of August it was found causing a leaf spot (Pl. I, Figs. A and B) on Crystal flax in a localized area in the University plots. These spots were brought to my attention by E. J. Hawn, student assistant.

The foregoing observations suggest that the organism has been present in Saskatchewan for some time, possibly on another host. *C. olivaceum* was reported by Wollenweber and Hochapfel (6) in 1937, on the stems of flax from the Argentine. The damage it causes on flax is at present of minor importance,

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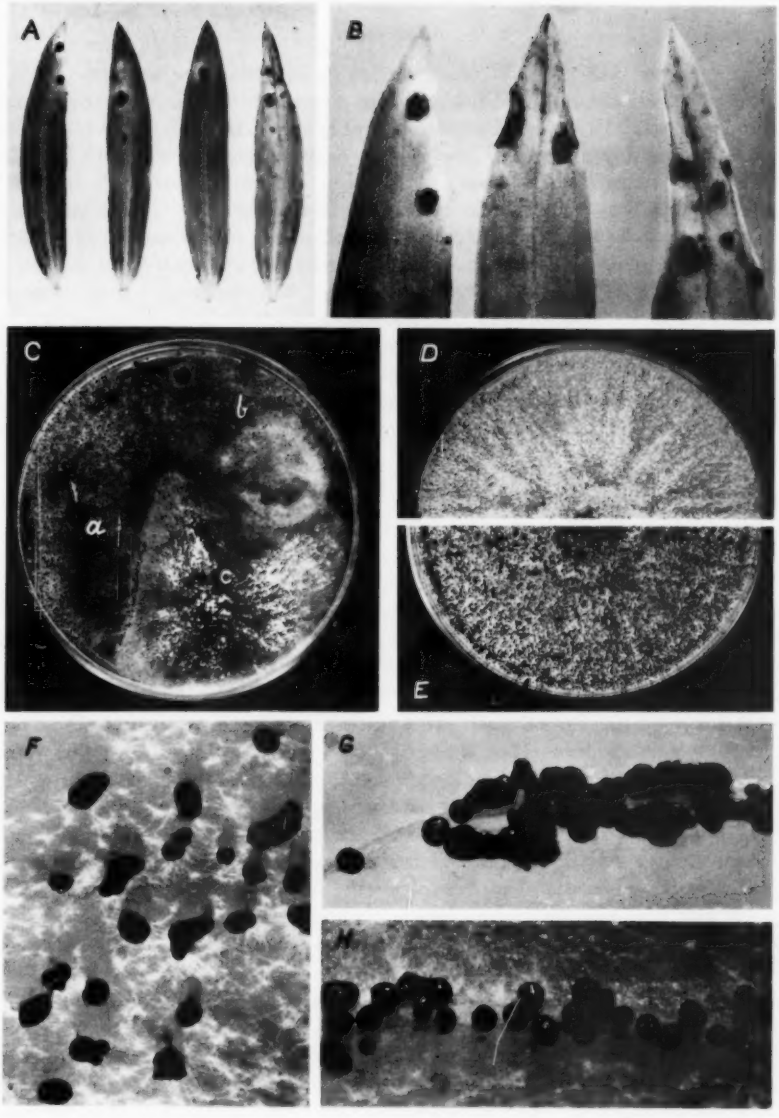
FIG. A. *Leaf spots caused by Coniothyrium olivaceum on Crystal flax from field collections; natural size.* FIG. B. *Leaf spots enlarged 3¼ times.* FIG. C. *Portions of disinfected, blighted flax seedlings on malt-extract agar showing at a, C. olivaceum; b, C. olivaceum and Rhizoctonia Solani; and c, R. Solani.* FIG. D. *A seven-day culture on malt extract agar.* FIG. E. *The same after 32 days showing numerous pycnidia.* FIG. F. *Pycnidia on a six weeks' culture on malt-extract agar; × 10.* FIGS. G *and* H. *Old isolation plates showing mature pycnidia on portions of flax seedlings; × 10.*


A
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C
a
b
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H

but its behaviour during 1945 and 1946 in Saskatchewan warrants the publication of this paper.

Isolations

The pathogen was obtained in culture from blighted flax seedlings from Scott in 1945, and from Saskatoon and Hudson Bay Junction in 1946; from lesioned cotyledons from Nipawin in 1946; from 12 out of 32 flaxseed samples selected for their low germination at the Seed Laboratory, Plant Products Division, Saskatoon; from leaf lesions on Crystal flax in August, 1946; and from one sample of sweet clover (*Melilotus alba* Desr.) seed where it was present as a trace only. The calcium hypochlorite method of disinfection as described by Wilson (5) was used, the seed being soaked, with intermittent shaking, in the stock solution for 10 min., and then plated out. Immersion for one to two minutes in the alcohol–mercuric chloride mixture of Machacek and Greaney (2), followed by three rinses in sterile water, also proved satisfactory. The fungus was grown in flasks of crushed oat hulls (sold as 'Vim' stock feed) for large-scale greenhouse and field tests. Spore suspensions were readily secured from cultures on the ordinary laboratory media, where it fruited profusely (Pl. I, Fig. E).

The Disease

C. olivaceum, besides being moderately pathogenic on flax, also caused moderate seedling blight on *Linum grandiflorum* Desf. in artificial inoculations. Both the sweet clover and flax strains were weakly parasitic on sweet clover in artificial inoculations. No definite parasitic ability was shown towards alfalfa (*Medicago sativa* L.), brome grass (*Bromus inermis* Leyss.), crested wheat grass, Argentine rape (*Brassica Napus* L.), sunflowers (*Helianthus annus* L.), or wheat (*Triticum aestivum* L.).

The disease on flax seedlings is of the same damping-off and blighting type commonly caused by *Rhizoctonia Solani* (3 and 4, p. 21), with *Pythium* and *Fusarium* spp. sometimes also associated. Affected seedlings usually dry up and are commonly buried by soil during subsequent spring rains. It is probable, therefore, since the parasite is internally seed-borne and may be isolated from diseased cotyledons (*vide supra*) that it develops pycnidia on the cotyledons of seedlings from which spores are disseminated for later leaf infections (Pl. I, Figs. A and B); these, in turn, supply inoculum for boll and seed infections. No pycnidia have been observed on seed from naturally infested samples, but they develop readily on infected seeds plated on nutrient media.

The leaf lesions appear to be confined to the top third of the leaf, being more common near or at the plane or straight margin of the leaf than near the convex margin (Pl. I, Figs. A and B). The spots are subspherical to oval with fairly regular margins, brown to dark brown in colour, and slightly roughened, especially in older lesions where pycnidia have formed. Adjoining areas soon turn yellow. Affected leaves fall off prematurely.

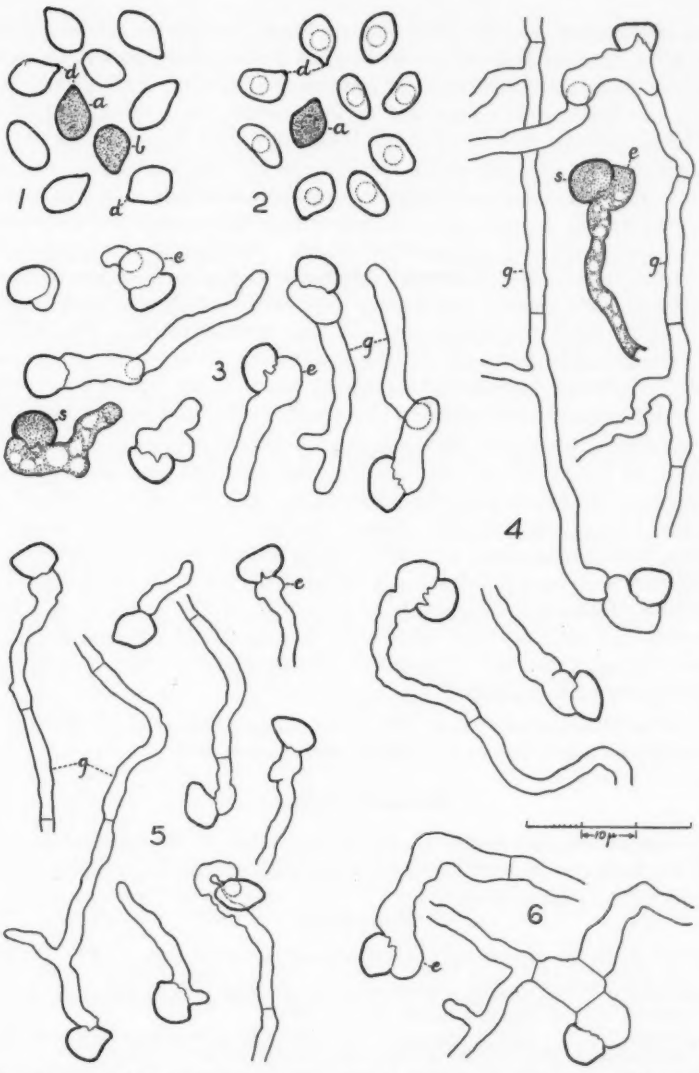
The damage caused to flax is of minor importance at the present time. If weather conditions favour heavy seed infection in the bolls, a slight reduction

in seedling stand might be expected when such seed is sown. The diseased areas on leaves were relatively smaller than diseased areas caused by pasmo (*Sphaerella linorum* Wr.) or browning (*Polyspora Lini* Laff.). Defoliation appeared to be hastened, but the damage caused is considered negligible especially in the light of Klages's experiments (1) on the effect of defoliation at various stages of growth on the yield of flax.

The Pathogen

The pathogen develops readily on cornmeal, malt extract, and potato-dextrose agars when naturally infested seed and seedlings, suitably surface sterilized, are plated in the usual manner (Pl. I, Fig. C, *a* and *b*). The growth is at first a dull, creamy white, with a mat surface; later, the mycelium becomes slightly aerial and orange-coloured in patches. Slight variations in mycelial densities give a radiating aspect to the growth on an agar plate (Pl. I, Fig. D). After about five days pycnidia begin to form, scattered indiscriminately over the surface of the agar (Pl. I, Fig. E). These are black, subspherical to irregular, and single or coalesced in groups (Pl. I, Fig. F) from which two or three ostioles may arise from a single chamber. The ostioles are apical or slightly to one side. Under high moisture conditions short, black columns of conidia may ooze through the ostioles, finally enveloping the pycnidia in a shining, viscid, black mass. Pl. I, Figs. G and H show pycnidia that have developed on portions of naturally infested flax seedlings after disinfecting and plating in the customary manner; in Fig. H, the seedling is more submerged in the agar than in Fig. G.

The majority of pycnidia fall within a diameter range of 250 to 350μ, with extremes of 180 and 600μ. The conidia are one-celled, thick-walled, brown to olivaceous, angularly ovate to napiform in surface view and flattened elliptical in side view (Figs. 1 and 2), measuring $7.5-10\ (11.5)\mu \times 4.2-7.5\mu$, with an average of $9.1 \times 6.0\mu$. In water mounts the contents are finely granular or slightly globular; in lactophenol the contents appear pale olivaceous brown, homogeneous or very slightly granular with a darker brown, spherical central globule. Certain light conditions frequently produce a dark-area effect or spot in the cell wall at the narrow, sometimes truncate, end (Figs. 1 and 2, *d*). Wollenweber and Hochapfel (6) record the occasional presence of a single septum in spores prior to germination, but no septa have been observed in the Saskatchewan form in germination studies. Germination occurs readily. The germ tube ruptures the cell wall and soon forms a characteristic swelling adjacent to the spore (Figs. 3, 4, and 5, *e*). Both the swelling and the hyphae are more robust on nutrient media (Figs. 3, 4, and 6) than in water or on plain agar (Fig. 5). In 24 hr., mycelial development is from three to six times as copious as that shown in Figs. 4 and 5. Radial growth rate is 11 mm. on cornmeal agar, 12.5 mm. on malt-extract agar, and 15 mm. on potato-dextrose agar in 24 hr. at 22° C.



FIGS. 1 to 6. *Coniothyrium olivaceum.* FIG. 1. *A water mount of spores from malt-extract agar; a, contents homogeneous or finely granular; b, contents slightly globular; d, dark spot in cell wall under certain light conditions.* FIG. 2. *A lactophenol mount showing a single globule in each spore; a, contents homogeneous.* FIG. 3. *Germination on cornmeal agar after 12 hr.; s, spore; e, enlargement, and g, germ tube.* FIG. 4. *Germination on cornmeal agar after 24 hr.* FIG. 5. *Germination on plain agar after 24 hr.* FIG. 6. *Germination on malt extract agar after 24 hr. Magn.* × *800. Drawn with the aid of a camera lucida.*

Pathogenicity

Pale reddish-brown, necrotic lesions are formed on the roots of seedlings on moist filter paper in Petri dishes when discs from culture plates are used as inoculum. Complete inhibition of germination is rare. Flax grown in the greenhouse during the winter and spring months in sterilized soil in pots inoculated with oat-hull inoculum showed slight stunting, with one to three seedlings usually, in a pot of 10, becoming sickly and finally blighting completely. *C. olivaceum* was re-isolated from such seedlings. In a similar test conducted during the summer, no outstanding difference could be observed between inoculated and uninocuiated plants. No disease developed in 1946 field plantings made from naturally infested 1945 seed or from inoculations of Redwing, Royal, Victory, and Viking flax with oat-hull inoculum scattered in the seed rows. The soil was dry at the time of sowing, with moisture conditions below normal until about mid-summer. The leaf infections found on Crystal followed a wet period in August; however, the infestation remained light and localized owing probably to subsequent dry atmospheric conditions.

In general, information on ecological conditions favouring the disease is meagre. Of the 12 infected seed samples from the 1945 crop, nine came from the park zone and three from the open prairie, possibly indicating that higher moisture favours the disease. There was an average of 8% seed-borne infection with a range from 1 to 32%. It is probable that the organism also overwinters in the pycnidial stage in infested leaves. According to Wollenweber and Hochapfel (6) pycnidia are formed on the stems of flax in the Argentine, but none could be found at Saskatoon on the stems of the Crystal flax having leaf lesions in late August. Dry atmospheric conditions doubtless prevented their development.

The observations recorded in this paper suggest that seed disinfection and crop rotation should serve as practical control measures.

Acknowledgment

My thanks are due to Dr. G. R. Bisby, Imperial Mycological Institute, Kew, England, for identifying the fungus and for drawing my attention to Reference 6.

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